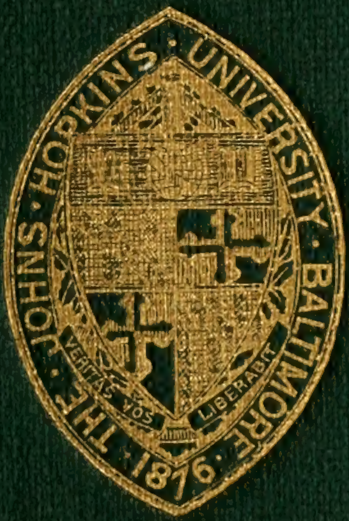
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The Use of Tissue in Broth in the Production of Diphtheria Toxin

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AND
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THE USE OF TISSUE IN BROTH IN THE PRODUCTION OF DIPHTHERIA TOXIN

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The production of diphtheria toxin regularly and of sufficiently high potency has long been a matter of primary importance. Although diphtheria toxin was among the first of the bacterial products to be discovered, but little is now known as to its composition or the basic substances from which it is produced. The well known uncertainty of toxin production and the seasonal variation in potency (MacConkey [1]) emphasize the importance of finding a method of producing toxin regularly and of satisfactory strength. A study of the literature on the nature of diphtheria toxin fails to supply much definite information.

An excellent résumé of the literature on the nature of this substance is contained in the paper by Robinson and Rettger.[2] It is of interest to mention a few of the conclusions of investigators having some bearing on the work which we are to report. It has been generally agreed that peptone was the important factor in enabling the diphtheria bacilli to produce toxin, Hida[3] believing that the secondary proteoses were the important constituents. Hitchens[4] and others found that simple peptone solution does not support the production of toxin. Dzierzgowski and Rekowski[5] believed that the toxin was a combination of certain bases and albumoses, as no toxin is obtained until the medium turns alkaline. The latter point was recently confirmed by Bunker.[6] The work of Hadley[7] on the production of toxin from protein-free mediums is open to criticism, since a whole culture and not a bacterial free filtrate was used for toxicity tests. Spronk[8] replaced meat infusion by an extract of brewer's yeast and claimed that he obtained stronger toxin and greater regularity in its production. Smith[9] found that the oxygen supply was an important factor and observed that 0.2% dextrose was favorable to toxin production on account of its acceleration of growth. No acid was formed from glycogen.

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[1] Jour. Hygiene, 1912, 12, p. 507.

[2] Jour. Med. Res., 1917, 36, p. 357.

[3] Ztschr. f. Hyg., 1908, 61, p. 273.

[4] Jour. Med. Res., 1905, 13, p. 523.

[5] Quoted from Robinson and Rettger.

[6] Jour. Bacteriol., 1919, 4, p. 379.

[7] Jour. Infect. Dis., 1907, Supplement 3, p. 95.

[8] Ann. de L'Inst. Pasteur, 1898, 12, p. 701.

[9] Jour. Exper. Med., 1899, 4, p. 373.

In view of the lack of Witte peptone, the former standard for toxin production, it seemed desirable to utilize only American peptone, especially since such a product would be constantly available. The proteose peptone manufactured by the Digestive Ferments Company and the peptone of the Parke-Davis Company have given uniformly good results with the method to be described. None of the other peptones tested gave as favorable results.

The medium used by us is essentially the "hormone" broth of Huntoon[10] and is prepared as follows: Finely ground lean veal is infused in twice its weight of water and is stored in the icebox for from 18 to 24 hours. It is then filtered through a wire gauze and heated to 80 degrees for 5 minutes. The infusion is thoroughly cooled and any fat present is removed from the surface. Two per cent. peptone and 0.5% sodium chlorid are added. The medium is brought to the boiling point and held there for 5 minutes. It is then titrated, using phenolphthalein as an indicator, to from neutral to 0.3% acid and again boiled 5 minutes. If the reaction is found to be correct the broth is filtered through wire gauze and the sediment allowed to settle. If necessary, the reaction is again corrected before filtration. The medium is distributed in 125 c c amounts in 300 c c. Florence flasks and sterilized 15 minutes at 15 pounds pressure. The reaction of the medium and its relation to toxin production is discussed in detail in another section.

In view of the fact that Bull and Pritchett[11] found that B. welchii produced more toxin in the presence of fresh sterile tissue, it was considered advisable to determine the effect on diphtheria toxin production of adding tissue to broth prepared in the manner already described. Various tissues from the rabbit and guinea-pig have been used, but guinea-pig liver tissue gives by far the best results. Liver tissue from guinea-pigs which have been negative to toxin tests are satisfactory, as no difference in results has been noted between tissue derived from these animals and that from normal pigs. The liver tissue was introduced into the flasks of broth in the following manner: A large guinea-pig was killed by a blow on the head or at the base of the skull and plunged into a 0.5% lysol solution for a few minutes. The body cavity was then asceptically opened and a mass of liver slightly larger than a 25-cent piece was introduced into each flask of 125 c c of medium. The liver tissue was not exsanguinated. Freshly drawn guinea-pig blood neither enhances nor inhibits the production of toxin. It seemed desirable to use guinea-pig tissue in order to avoid foreign protein toxicity in the diphtheria toxin tests. It has been found that when guinea-pig liver tissue is removed with care, according to the technic just described, it is nearly uniformly sterile for less than 1 out of 10 pigs appears to be infected. The liver tissue was transferred directly from the animal into the flasks of sterilized mediums and inoculated immediately with the culture of diphtheria bacillus because tissue which has stood in mediums in the incubator to test its sterility loses its power of enhancing toxin production. The reason for this loss of enriching property is not clear. Contaminations of the mediums from infected tissue or other sources can be detected in smear preparations at the time of testing the toxin and also by the odor of the broth.

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[10] Jour. Infect. Dis., 1918, 23, p. 169.

[11] Jour. Exper. Med., 1917, 26, p. 119.

On account of the favorable results obtained by the addition of sterile liver tissue to broth, the effect of adding a water extract of beef liver tissue to broth in place of the tissue was tried but with negative results. Infusion broth prepared from beef liver instead of veal does not give toxin production comparable with the ordinary infusion broth to which guinea-pig liver has been added. Two explanations may be advanced for these results: (1) there may be some physical or mechanical reaction with the freshly excised liver tissue or (2) the essential substance is destroyed by sterilizing the liver infusion.

Inoculation of the tissue broth was made from fresh moist 24-hour cultures on Loeffler blood serum and luxuriant pellicle formation always resulted within 30 hours, provided the tissue was sterile. When the tissue was not sterile pellicle formation was not only greatly retarded, but the piece of tissue usually rose to the surface and floated, whereas sterile tissue invariably remained at the bottom of the flask. The Park-Williams strain No. 8 was used in all tests unless otherwise noted.

TABLE 1

THE EFFECT OF THE INITIAL REACTION ON TOXIN PRODUCTION

Initial Reaction	Incubation	Dilution of Toxin		Remarks
		0.01	0.005	
Neutral	4 days	Not tested	Pig died after 3 days	Heavy pellicle
Plus 0.1	4 days	Pig died after 2 days	Pig died after 2 days	Heavy pellicle in 36 hours
Plus 0.3	4 days	Not tested	Pig died after 2 days	Good pellicle
Plus 0.45	6 days	Pig died after 6 days	Pig died after 7 days	Pellicle formed slowly
Plus 0.5	5 days	Negative	Negative	Reaction in 0.01 pig
Plus 0.5	7 days	Pig died after 3 days	Negative	Pellicle formed in 48 hours
Plus 0.8	8 days	Negative	Not tested	Pellicle formed in 36 hours
Plus 1.0	8 days	Negative	Not tested	Poor pellicle formation
Neutral (control)	6 days	Negative	Negative	0.1 toxin produced

Since the purpose of this study was to find a method that would assure the regular production of toxin of usable strength rather than one which would supply an occasional high titer toxin interspersed with many nontoxin producing starts, a minimum lethal dose of 0.005 was set as a standard. Tests of considerably higher dilutions of tissue enriched broth were occasionally made and the results in most cases were positive. While not all the toxins produced were tested at a strength of 0.003 there is every evidence to show that most of them were at least of that titer while several had a titer greater than 0.0025. Broth prepared with Parke-Davis peptone and not enriched with liver tissue usually produced a low potency toxin with occasional toxins of a titer up to 0.005. Proteose peptone without tissue produced a 0.005 toxin or one of somewhat higher strength with greater regularity but in our hands 25% of the lots of medium prepared with this peptone failed to produce a 0.005 toxin.

As Bunker[6] has pointed out, the initial reaction of the broth determines to a considerable extent the potency of the toxin produced, as well as the character and speed of pellicle formation. Table 1 gives the initial reactions of different lots of broth varying from neutral to 1% acid to phenolphthalein.

Parke-Davis peptone was used in the preparation of each lot shown in this table. Tissue was used in the broth in each case unless otherwise indicated.

The reactions to phenolphthalein were checked by hydrogen-ion readings by the colorometric method and mediums showing reactions to phenolphthalein ranging from neutral to plus 0.3% fall within or near the limits indicated by Bunker.[6] It seems worthy of notice that equally satisfactory results are obtainable when the reaction of diphtheria toxin broth is adjusted by either the phenolphthalein or hydrogen-ion method. Table 1 indicates that in broth to which tissue has been added the most favorable initial reaction range to phenolphthalein is from neutral to plus 0.3%. As the reaction approaches plus 1.0%, toxin production becomes more and more irregular and finally ceases entirely. When toxin is formed in mediums with acid reactions of more than plus 0.3% to phenolphthalein the titer is lower than in mediums prepared at the same time and from the same veal infusion but with a reaction below plus 0.4%.

The initial reaction also appears to bear a fairly direct relation to the rate and luxuriance of pellicle formation for with mediums ranging from neutral to plus 0.3% a heavy, firm pellicle is formed within 24 hours and increases in thickness for about 3 days, after which it appears to remain constant. With a higher concentration of acid, pellicle formation is greatly retarded and the pellicles when finally formed are frequently incomplete or extremely thin and easily destroyed by slight agitation of the medium. The same conditions regarding the effect of the initial reaction of the medium on toxin production and pellicle formation seem to apply to proteose peptone as table 2 demonstrates.

TABLE 2

THE EFFECT OF THE INITIAL REACTION ON TOXIN PRODUCTION

Initial Reaction	Incubation	Dilution of Toxin		Remarks
		0.01	0.005	
Neutral	4 days	Pig died after 3 days	Pig died after 4 days	Heavy pellicle
Plus 0.2	4 days	Pig died after 2 days	Pig died after 5 days	Heavy pellicle
Plus 0.5	7 days	Negative	Negative	Slow pellicle formation
Plus 0.8	7 days	Not tested	Pig died after 8 days	Slow pellicle formation
Neutral (control)	4 days	Pig died after 4 days	Negative	Good pellicle formation
Neutral (control)	5 days	Not tested	Pig died after 2 days	Heavy pellicle

In testing the toxins shown in tables 1 and 2 due account was taken of the fact brought out by Bunker[6] that when the initial reaction is acid a longer incubation period must be allowed for the reversal to the alkaline reaction. It appears, however, that even when incubation is prolonged toxin is of low potency if the initial reaction varies to any great extent from the optimum.

Although the initial reaction of broth is of the greatest importance, it is equally necessary that the reaction become markedly alkaline if a high titer toxin is to be produced. Bunker[6] has already pointed out this fact and experiments with broth enriched with liver tissue confirm his observations. A tissue enriched flask seldom shows a reaction more alkaline than control flasks to which no tissue has been added. In the great majority of cases the reactions of the control flasks increase in alkalinity at a more rapid rate after the third day or sometimes the fourth day of incubation. This point is discussed later. Table 3 shows the initial reaction, the toxin production and the reaction at the time of testing of a lot of broth prepared with Parke-Davis peptone.

The interesting fact is brought out by this table that although the final hydrogen-ion reaction may be in a range where toxin is produced, nevertheless, if the initial reaction is too alkaline there is no toxin production. Here again the period of incubation was taken into account in order that sufficient time might be allowed for toxin production. When the reaction before testing has reached a value of P_H 8.0 one may expect to find a potent toxin in so far as this detail is concerned. It appears evident that toxin is only produced in broth which has a final reaction of at least P_H 8.0 and that stronger toxins usually have a more alkaline reaction up to P_H 8.3. A favorable reaction at the time of testing is not, however, an indication that toxin has been produced, for many lots of mediums without tissue enrichment had hydrogen-ion reactions within ranges where high titer toxins would be expected but with negative tests in guinea-pigs. It is evident that while the initial and final reaction of the medium has a direct bearing on toxin production other factors must also be favorable if the medium is to yield toxin.

TABLE 3

THE EFFECT OF INITIAL AND FINAL REACTION ON TOXIN PRODUCTION

Initial Reaction		Toxin Production		Reaction before Filtration, P_H
Phenolphthalein	P_H	0.01	0.005	
—0.1	7.9	Negative	Negative	8.0
Neutral	7.6	Died after 4 days	Died after 4 days	8.0
Neutral	7.6	Died after 4 days	Died after 4 days	8.0
Neutral	7.7	Not tested	Died after 3 days	8.2
Plus 0.4	7.3	Died after 3 days	Died after 3 days	8.3
Plus 0.4	7.2	Not tested	Died after 3 days	8.3
Neutral (control)	7.3	Died after 11 days	Negative	8.2
Neutral (control)	7.4	Died after 2 days	Died after 3 days	8.1

The addition of sterile liver tissue to broth appears to reduce the necessary period of incubation considerably. Most investigators have reported that incubation of not less than 6 days and usually longer is required. According to Bunker[6] this period is closely bound up with the progressive change in the reaction of the broth into an alkaline range. While this may be true, the constituents of the broth and particularly those substances that reduce the lag period and greatly enhance rapid growth, shorten the necessary incubation period for the production of a favorable alkaline reaction. When tissue is added to broth the optimum range of alkalinity appears to extend over a longer period of time than in unenriched mediums. Apparently the liver tissue has a buffer action independent of the peptone but this point has not been determined absolutely.

Diptheria bacilli grow with much greater rapidity in broth to which a piece of sterile liver tissue has been added than in unenriched mediums. Pellicle formation is far more luxuriant and the pellicle shows as a white film in from 18 to 24 hours, while a pellicle of equal strength and thickness is not formed on normal broth until from 30 to 36 hours have elapsed. The increase in strength and thickness of the pellicle after it is formed is more rapid when tissue has been added and at any time during incubation the enriched broth presents a more favorable pellicle for toxin production than control flasks of the same lot of medium. It is natural to expect that when growth and luxuriant pellicle formation are so rapid less time would be required for the production of a toxin of reasonably high potency. Table 4 gives the toxin production over a series of days of flasks filled with the same lot of broth and inoculated under as nearly the same conditions as possible. Parke-Davis peptone was used in the preparation of this broth and the reaction was neutral to phenolphthalein.

TABLE 4

Relation of the Period of Incubation to Toxin Production

Period of Incubation	Dilution of Toxin	
	0.01	0.005
4 days	Not tested	Died after 4 days
4 days (without tissue)	Negative	Negative
5 days	Not tested	Died after 5 days
5 days (without tissue)	Negative	Negative
6 days	Not tested	Died after 3 days
6 days (without tissue)	Negative	Negative
7 days	Not tested	Died after 2 days
8 days	Died after 2 days	Died after 3 days
8 days (without tissue)	Died after 2 days	Died after 3 days

TABLE 5

Relation of Initial Reaction, Rate and Potency of Toxin Production and Final Reaction

Period of Incubation	Dilution of Toxin		Final Reaction
	0.01	0.005	
3 days—tissue	Not tested	Died after 2 days	P_H 8.0
3 days—control	Not tested	Negative	P_H 7.9
4 days—tissue	Not tested	Died after 4 days	P_H 8.1
4 days—control	Not tested	Negative	P_H 8.1
5 days—tissue	Not tested	Died after 2 days	P_H 8.1
5 days—control	Died after 2 days	Died after 2 days	P_H 8.2
6 days—tissue	Not tested	Died after 3 days	P_H 8.2
6 days—control	Not tested	Negative	P_H 8.3
7 days—tissue	Not tested	Negative	P_H 8.3
7 days—control	Not tested	Negative	P_H 8.4

This series of tests was selected because one of the flasks of broth without tissue enrichment produced a 0.005 toxin and this fact allows a comparison with tissue enriched mediums in relation to speed of toxin production. Unfortunately the final reaction of this series of tests was not recorded, but similar series in which the P_H value was taken showed that broth enriched with sterile liver tissue attains a favorable alkaline reaction usually on the third day provided the initial reaction ranged from neutral to plus 0.3% to phenolphthalein as shown in table 5. The control flasks filtered on the fourth, fifth and sixth days did not contain a 0.01 toxin but on the eighth day a 0.005 toxin had been produced. It is evident from this table that broth without tissue enrichment requires longer incubation than flasks filled with the same lot of medium to which sterile pieces of liver tissue have been added. Table 5 brings out the relation between the initial reaction the rate and potency of toxin production and the final reaction. In this lot of broth proteose peptone was used and the initial reaction was neutral to phenolphthalein or P_H 7.7. A flask with tissue and a control were inoculated for each of the 5 days and tested as indicated.

This table presents clearly the advantage derived from adding tissue to broth which produces toxin. When the initial reaction is in the correct range for toxin production tissue enriched broth reaches a favorable alkalinity usually on the third or fourth day and the reaction continues advantageous over a considerable period of time. Furthermore, this table confirms the results recorded in table 4, for toxin production was more rapid in broth containing tissue than in the same lot of medium unenriched. When tissue is added to broth there appears to be less danger of collecting the toxin at an incorrect period of incubation since toxin is frequently found in tissue free broth on only one or two days during the whole course of the experiment.

The effect of an initial acid reaction on the rate and potency of toxin production is brought out in table 6. In this lot of broth proteose peptone was used and the initial reaction was plus 0.4 to phenolphthalein or P_H 7.2. A flask with tissue and a control were inoculated for each of the 5 days.

TABLE 6

THE EFFECT OF AN UNFAVORABLE ACID REACTION ON TOXIN PRODUCTION

Period of Incubation	Dilution of Toxin		Final Reaction
	0.01	0.005	
4 days—tissue	Negative	Negative	P_H 8.0
4 days—control	Negative	Negative	P_H 7.9
5 days—tissue	Died after 2 days	Negative	P_H 8.2
5 days—control	Negative	Negative	P_H 8.1
6 days—tissue	Not tested	Died after 6 days	P_H 8.3
6 days—control	Negative	Negative	P_H 8.3
7 days—tissue	Died after 2 days	Died after 3 days	P_H 8.3
7 days—control	Negative	Negative	P_H 8.4
8 days—tissue	Not tested	Died after 7 days	P_H 8.4
8 days—control	Not tested	Died after 6 days	P_H 8.3

The same point is again brought out in this table as in table 3. The fact that the final reaction has reached a favorable degree of alkalinity is no guarantee that toxin has been produced. The effect of liver tissue on broth of poor toxin producing qualities is well shown in table 6. While the control flask of the eighth day of incubation contained a 0.005 toxin of poor quality, the flask with tissue enrichment on the seventh day showed a good quality, 0.005 toxin and one of the same strength but poorer quality on the sixth day. This table again confirms the results recorded in table 4, since toxin was produced with greater rapidity in broth containing sterile tissue.

Since the purpose of this study was to devise a method of producing diphtheria toxin regularly, little attention has been given to the exact potency of the product provided it was at least 0.005. Some of the control lots of broth, particularly that prepared with proteose peptone, produced toxins of a strength as high as 0.0033 and even 0.0025. In such cases the corresponding flask of broth with tissue added gave an equally high titer and within a shorter period of time. Occasionally a broth with tissue enrichment was tested at 0.0033, and in most cases was positive, although the control flask frequently did not contain a 0.01 toxin. Tests have been made on 18 lots of tissue enriched broth prepared with proteose or Parke-Davis peptone, the initial reaction ranging from neutral to plus 0.3% to phenolphthalein. In each of these lots a 0.005 toxin was obtained from flasks incubated from 3-7 days, the optimum period of incubation appearing to be about 5 days. Broth has been prepared by other methods than that previously described, but with unsatisfactory results. Flasks of mediums in which tissue was incubated 24 hours to test for sterility before inoculation with the culture of B. diphtheriae failed to produce toxin. Evidently, the liver tissue must be absolutely fresh to be of value in enhancing toxin production. Similar results were obtained by Bull and Pritchett [11] in their study of the toxin production of B. welchii.

Several strains of B. diphtheriae have been tested for toxin production by this method and encouraging results have been obtained. These cultures were isolated from active or convalescent cases, and had been cultivated on Loeffler blood serum slants for from 1-12 months. They had never been grown in broth up to one week before the tests were made when they were planted in tubes of "Hormone" broth to which small pieces of liver tissue had been added to encourage pellicle formation. After transferring a few times, pieces of the pellicles were transplanted onto the surface of broth in flasks after the tissue had been added. Eleven strains of B. diphtheriae were tested in this manner, of which but one failed to produce toxin. Eight of the remainder produced a 0.02 toxin, one had a strength of 0.006, while one killed in a dilution of 0.004. These tests still further support the belief that the addition of liver tissue to broth is of great value in the production of toxin.

The explanation of the effect of tissue on toxin production is extremely difficult owing to the lack of knowledge of the chemistry of tissue, especially in relation to the metabolism of bacteria. Certain theories may be advanced, however. Liver tissue, owing to its high glycogen content, furnishes an easily available food for the bacteria. Since B. diphtheriae does not form acid from glycogen no effect on the reaction of the medium results, even though it is rapidly attacked. Again, the tissue may absorb some of the oxygen in solution in the broth, thereby causing the diphtheria organisms to grow in thick pellicle formation on the surface in order to obtain an adequate supply of oxygen. The fact that only fresh tissue is of value indicates that this theory may be at least a partial explanation of the favorable results, for tissue which has stood for twenty-four hours in uninoculated broth has probably reached its limit of oxygen absorption. The surface of such a medium, unprotected by pellicle formation, is still in direct contact with the oxygen of the air which the broth probably continues to absorb. Finally, liver tissue may supply in extra quantities substances similar to those found by Spronk[8] in yeast cells, which he claimed to be essential to toxin production. Since glandular tissue is particularly rich in vitamins, the presence of these substances in liver tissue may have an important effect on toxin production. Perhaps no one of these theories completely explains the favorable action of liver tissue, but rather a combination of them. The presence of both glycogen and accessory substances in the tissue seems to us, however, to account for the rapid growth and regular toxin production in broth which has a favorable reaction. In a study of the hemotoxin production of streptococci we have found that there is in fresh tissue some substance of great delicacy essential for the elaboration of hemotoxin. In many respects there appears to be a similarity between the substances from which diphtheria toxin and the hemotoxin of streptococci are derived. The presence of glycogen and accessory substances in tissue seems to us, however, to be presumptive evidence that they play an important part in the rapid and regular toxin production.

No toxin has been produced by this method on a commercial scale owing to a lack of proper facilities. It is therefore impossible to state whether or not the trouble and expense of adding liver tissue to broth would be warranted under commercial conditions when large amounts of toxin must be produced. These observations, however, may be of value in suggesting new lines of attack on the question of the constitution of diphtheria toxin.

CONCLUSIONS

Diphtheria toxin can be produced regularly in broth to which pieces of sterile guinea-pig liver have been added. The medium must be inoculated immediately after the addition of the tissue.

Broth prepared with certain American peptones gives satisfactory results when enriched with liver tissue.

The most favorable initial reaction of the broth ranges from plus 0.3% to neutral to phenolphthalein.

The broth at the time of testing should have a reaction ranging from P_H 8.0 to P_H 8.3, although a favorable reaction is not the only essential for toxin production.

The addition of liver tissue reduces the necessary period of incubation.

Strains of B. diphtheriae other than Park-Williams No. 8 have produced a toxin of high potency by this method.

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THE PRECIPITIN REACTION IN THE DIAGNOSIS OF GONOCOCCUS INFECTIONS

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The laboratory diagnosis of gonococcus infections is at present accomplished by (1) microscopic examination of pus, (2) the cultivation of the gonococcus from infected discharge or tissue and (3) the complement fixation test upon the blood of the patient.

Microscopic examination is the most extensively used by reason of its simplicity and the rapidity with which results may be obtained. The classical picture is one in which Gram-negative, kidney-shaped diplococci are contained within polymorphonuclear leucocytes. The diagnosis by this method may be readily arrived at in the examination of the urethral discharges in recent infections in the male but becomes more uncertain after the acute stage is passed. In vaginal specimens the abundance of bacterial forms frequently makes a satisfactory report impossible. Organisms resembling the gonococcus in morphology and staining reactions are sometimes found and failure to find the gonococcus is not conclusive evidence that the patient is not harboring this organism. Cultivation of the gonococcus is often difficult owing to the delicacy of the organism. When there is a considerable number of different organisms present as in the case of vaginal specimens, cultural methods are impractical. The complement fixation test seems to be satisfactory in only a small percentage of infections, these being as a general rule in the chronic stage. In view of these difficulties the development of some other method of diagnosis may be of interest.

The work of Krumwiede and Noble (1918) on the diagnosis of pneumococcus infections by the precipitin reaction, that of

Vincent and Bellot (1914) and one of us, Robinson, (1919) on the diagnosis of meningococcus infection by the same method led us to try the value of this reaction in the case of gonorrhea. The literature on the subject is meagre. Watabiki (1918) attempted to diagnose infection by using the serum of the patient with a known gonococcus autolysate. He was able to get a precipitin reaction in a few instances, these being old or chronic cases. No strain specificity was observed. Torrey (1907) has found some strain specificity by using gonococcus autolysates and immune rabbit serum.

In the accomplishment of the precipitin test the preparation of a suitable precipitinogen is of the first importance. Krumwiede boiled pneumonic sputum in a 5 per cent hypochlorite solution. Torrey and Watabiki incubated gonococci in a 0.15 per cent sodium hydroxide solution and then carefully neutralized with acid. Our experience in producing precipitinogen by chemical means showed that the chemical reaction must be very carefully adjusted to the neutral point if false precipitin reactions are to be avoided. We found that if autolysis was permitted in distilled water or preferably in 0.85 per cent sodium chloride solution, a suitable preparation was obtained and in all the work herein reported this method has been used.

It was considered advisable to try the reaction of immune rabbit serum and the autolysate of gonococcus cultures. The cultures included old laboratory strains and others freshly isolated for this work. Animals were injected intravenously with single strains in gradually increasing doses at seven day intervals. Five or six injections sufficed to produce a serum giving a marked precipitin reaction in a dilution of at least one to twenty. The autolysate was prepared by washing off the growth on rabbit blood agar in 5 cc. of physiological salt solution and allowing it to stand in the incubator six hours. This suspension was then centrifuged until the supernatant liquid was perfectly clear. Clear serum was placed in serological test tubes and the clear autolysate stratified over the serum in equal amount, generally 0.25 cc. The tubes were then placed in a water bath at 37°C. for one hour. After the tubes have been cooled either at room temperature or in the ice-box the results are observed. A positive reaction is indicated by a cloudy white ring at the point of contact of serum and autolysate. The reading of the results is greatly aided by holding the tubes before an electric light. All strains of gonococci gave a positive reaction with immune serum and no differences were noted between the reaction of the homologous and heterologous autolysates so there seemed to be no strain specificity.

The autolysate for diagnostic purposes is obtained by moistening a sterile cotton swab in the suspected material and incubating in two cubic centimeters of physiological salt solution for six hours. The swab is then removed and the infusion is centrifuged until a perfectly clear supernatant liquid is obtained. Occasionally we find that a specimen especially of vaginal origin, remains opalescent in spite of continued centrifugation. We have not as yet been able to overcome this difficulty but the correct reading of the test can be obtained by comparing the density of the precipitated ring in the test tube containing immune rabbit serum with that of the control tube containing normal rabbit serum.

The procedure in brief is as follows: for each specimen to be examined 0.25 cc. of diluted clear serum from two immune and one normal rabbit are placed in serological test tubes. The clear extract of the specimen to be tested is layered over the serum in the same amount. The tubes are incubated for one hour at 37°C. and are allowed to cool before readings are made. Most of the results reported were obtained by the use of serum from rabbits injected with two strains. Two immune sera are used in the test in order to duplicate the results while the serum from a normal animal prevents reading any non-specific precipitation. In the case of some sera there appeared to be a false reaction when sterile salt solution was layered over the serum. This difficulty was overcome by diluting the serum with salt solution and centrifuging until it was perfectly clear. This diluted serum was used in both the test and control tubes in place of the undiluted serum. The dilution used in making the tests is one to two.

When it had been amply demonstrated that pure culture autolysates of gonococci always gave a positive precipitin test specimens of urethral discharge from males having known acute gonococcus infection were obtained. The favorable results from these cases encouraged us to attempt vaginal specimens and others offering a more difficult diagnosis. One hundred and forty-nine specimens have been examined and the results appear to be in accord with the history and clinical examination. The cases from which specimens were obtained fall clinically and by microscopical examination into eight groups. In all but the last group the specimens are from the urinary or genital tract.

Group I. Known positive cases: Those showing Gram-negative, kidney-shaped, intracellular diplococci. The patient gives a history of exposure to infection and the clinical diagnosis is gonorrhea.

Group II. Probably positive cases: The Gram-negative, kidney-shaped diplococci are inconstant or scarce. The history and clinical examination point strongly to infection with M. gonorrhoeae.

Group III. Doubtful cases: The history and clinical examination points to gonococcus infection but microscopical examination reveals no microorganisms or only bacillary forms.

Group IV. Cases under treatment: The clinical diagnosis of each case had been confirmed by microscopic examination of pus. Treatment had progressed so far that no gonococci appeared in the discharge.

Group V. Chronic cases: No treatment had been given for some time. Suspicious organisms are absent from the discharge but clinical symptoms indicate some local infection.

Group VI. Vulvo-vaginitis in children: The microscopic examination showed in each case Gram-negative, kidney-shaped diplococci contained within the leucocytes.

Group VII. Presumably negative cases: Vaginal swabs obtained from women under treatment for conditions other than gonorrhea.

Group VIII. Known negative cases: Pus collected from surgical cases in which the causative agent was known not to be M. gonorrhoeae.

A tabulation of the specimens examined in each group is shown in protocol I.

Group I in which gonococci were present gave positive reactions in each instance. The two cases reported as negative under group II were not positive microscopically. While the history might indicate gonococcus infection there is no proof that this is true. The large percentage of positives in this group, however, indicates a substantial correlation between clinical examination and the precipitin test. The same correlation of results is shown in group III. The results in groups IV and V point to the reliability of the precipitin test as compared with microscopic examination as these cases were microscopically negative. Our one negative result in this group may have indicated a complete cure. Group VI is of interest in view of the findings of Pearce (1915) that the type of gonococcus found in infants was distinct by the complement fixation test from the organism in adult infections. Group VII was made up of patients in a women's medical ward. The case which we found positive was afterward examined microscopically and found to harbor suspicious organisms. The specimens in group VIII comprised pus from a boil, osteomyelitis of the foot, neck abscesses, periostitis of the tibia with a negative Wassermann reaction and an alveolar abscess. In each case a staphylococcus or streptococcus was cultivated.

In order to still further test the accuracy of the precipitin reaction a more complete examination was made of eleven women under treatment for gonorrhea. Specimens were carefully taken from the cervix, urethra and vagina. Protocol II shows the results obtained.

While gonococci were demonstrated in only two of the cases a positive precipitin test was obtained from at least one source from each patient. In four, either the cervix or urethra gave a negative test but the vagina, exposed to infection from either source, was always positive. Where the vagina gave a positive test in the presence of a negative reaction from both cervix and urethra an infection of the vaginal wall itself may be assumed.

It has been recognized by considerable experimental work that the precipitin reaction will detect minute amounts of protein.

The test as applied to the diagnosis of gonorrhea depends upon the presence of the gonococcus protein and should be specific. The gonococcus is notably susceptible to autolysis and it is probable that in the case of a localized infection the products of autolysis might be present in secretions in which the organisms could not be demonstrated.

From these results the precipitin reaction seems to have an advantage over the microscopic examination in three respects, viz.: in testing specimens from cases where the diagnosis is doubtful, in examining specimens containing an abundance of microorganisms such as vaginal secretions and in determining the presence of infection in cases under treatment. The immune serum which we have used, for the most part produced by the use of two strains of gonococci, has seemed to react upon all infecting strains. It is possible that the dilution of serum used is not sufficient to distinguish strain specificity if such a condition does exist. Bacterial species which gave a negative reaction with gonococcus immune serum are M. albus, M. aureus, M. citreus, M. catarrhalis, Strep. hemolyticus, and B. coli. The meningococcus, however, gave a positive reaction when a concentrated pure culture autolysate was used but the gonococcus control autolysate continued to give a positive reaction for a considerable number of dilutions after the reaction with meningococcus autolysate had become negative. It is improbable, moreover, that meningococcus protein would be found in secretions in which the gonococcus was sought.

Work on a large number of cases is in progress to determine the value of the test as a routine measure. The number of cases presumably negative in our series is comparatively small and more are necessary to the establishment of the test on a firm basis. While the number of examinations made in some of the groups reported is not large, sufficient work has been done to warrant certain conclusions.

1. The present methods of diagnosing gonorrhea are inadequate since in many cases, clinically positive gonococci cannot be demonstrated in the discharge.

2. A positive precipitin test is obtained in all cases where the gonococcus can be found in the discharge.

3. A positive precipitin test is found in specimens from many patients whose history and clinical symptoms point to gonorrhea but in whose discharges the gonococcus cannot be demonstrated.

4. The precipitin test is of value in the diagnosis of vaginal and other specimens where the microscopical demonstration of gonococci in the discharge is difficult or impossible.

Material from patients was obtained from the United States Government Clinic, from the wards and dispensary of the Johns Hopkins Hospital, from the Maryland General Hospital and from private cases. Our thanks are due to the physicians and medical students through whose courtesy and assistance the specimens were collected.

Protocol I. Correlation of clinical condition and the precipitin test

GROUP	CLINICAL CLASSIFICATION	M. GONORRHEAE BY MICROSCOPICAL EXAMINATION	SEX	PRECIPITIN TEST		
				Positive	Negative	Total
I	Gonorrhea	Present	Female	19	0	61
			Male	42	0	
II	Probable gonorrhea	Few or inconstant	Female	11	1	22
			Male	9	1	
III	Doubtful	Absent	Female	7	0	10
			Male	2	1	
IV	Gonorrhea under treatment	Disappeared under treatment	Female	9	1	16
			Male	6	0	
V	Chronic gonorrhea treatment suspended	Disappeared under treatment	Female	4	0	6
			Male	2	0	
VI	Vulvo-vaginitis in children	Present	Female	7	0	7
VII	Non-—gonorrheal medical cases	Absent except perhaps in one case	Female	1	17	18
VIII	Non-—gonorrheal surgical cases	Absent	Not specified	0	9	9
						149

Protocol II. Determination of the location of infection

CASE NUMBER	CERVIX	URETHRA	VAGINA
1	Negative	Negative	Positive
2	Negative	Positive	Positive
3*	Positive	Positive	Positive
4	Negative	Positive	Positive
5	Not tested	Positive	Positive
6*	Positive	Positive	Positive
7	Positive	Negative	Positive
8	Negative	Negative	Positive
9	Positive	Negative	Positive
10	Negative	Negative	Positive
11	Positive	Positive	Positive

* Positive microscopically.

REFERENCES

Torrey, J. C.: J. Med. Research, 1907, xvi, 329.

Vincent and Bellot: Quoted from Besson, A., Technique Microbiologique et Serotherapique, 1914, p. 391.

Pearce, L.: J. Exp. M., 1915, xxi, 289.

Krumwiede, C., and Noble, W. C.: J. Immunol., 1918, iii, 1.

Watabiki, T.: J. Infect. Dis., 1918, xxii, 115.

Robinson, G. H.: N. Y. M. J., 1919, cix, 464.

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BIOLOGICAL AND PHYSICAL PROPERTIES OF THE HEMOTOXIN OF STREPTOCOCCI.

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The isolation of hemolytic streptococci from many different pathological lesions demonstrates their importance as pathogenic organisms. Various investigators point out two types of these organisms, one of which produces hemolysis only on blood agar, while the other produces also a hemolytic substance, or hemotoxin, in bouillon. Whether or not all laking of erythrocytes as exhibited on blood agar plates by different strains is the result of the same reaction is not clear. However, the ability to produce hemotoxin in bouillon seems to be a definite characteristic of certain strains of streptococci. Since hemolytic power is a biological function of most virulent streptococci, it is of interest to note the nature of the hemotoxin.

Frequent attempts have been made to separate hemotoxin from the organisms by filtration. The matter of filtration is of the first importance in showing whether the hemotoxin is in solution or is contained within the bacterial cells. Aronson (1902) states that hemotoxin can pass through a filter. Besredka (1901), using a Chamberland filter, noted a relation between the rate of filtration and the hemolytic strength of the filtrate and suggests that perhaps the active substance is retained in the pores of the filter. Ruediger (1903) recommends that the filter should be carefully selected, as too fine a filter removes the hemolytic property. Von Hellens (1913) and Nakayama (1919) were able to obtain hemolytic filtrates only with considerable loss of potency during the procedure. Maasen filters were used in their experiments. M'Leod (1911–12) and Braun (1912) were able to secure hemolytic filtrates by the use of Maasen filters. Lyall (1914) was not able to obtain hemolytic filtrates by the use of coarse Berkefeld filters.

Another method of separating the bacterial cells from the hemotoxin is by centrifugation. Levin (1903) reported that there was nearly 1,000 times as much hemotoxin in the sediment of a centrifuged tube of medium as in the filtrate of the same fluid. A comparison of the sediment and the supernatant fluid after centrifugation would seem to have more value, however. Schlesinger (1903) concentrated the streptococci by centrifuging and found that hemotoxin was liberated by crushing the cells.

Little is recorded regarding the chemical nature of the hemotoxin. Ruediger (1903) states that it is composed of a toxophore and haptophore group loosely bound together. Von Hellens (1913) found hemotoxin soluble in ether and extracted it by this method. Von Lingelsheim (1912) reports that hemotoxin is of the nature of an enzyme. Lyall (1914) is of the opinion that it is not an enzyme because the hemolytic power of his cultures was destroyed by exposure to chloroform and toluene, reagents not destructive to enzymes.

The culture medium used by different workers for the production of hemotoxin varies widely. M'Leod (1911–12) found that the addition of 15 to 20 per cent of horse serum to bouillon produced the most powerful hemotoxin. Nakayama (1919) added 50 per cent or more horse serum to broth. Von Hellens (1913), on the other hand, found that the addition of horse serum in amounts of more than 40 per cent tended to decrease the amount of hemotoxin produced. Lyall (1914) used a calcium carbonate broth to which 16 per cent ascitic fluid was added.

Owing to the contradictory results reported in the literature a repetition of much of the work on this subject seemed necessary. The object of the work reported in this paper was to determine if possible something of the nature of the hemotoxin by means of a study of its filterability, the effect of adsorptive agents, the effect of centrifugation and shaking, and the basic substances from which it is produced.

In all the work a standard culture medium was used consisting of bouillon with 1 per cent of Difco peptone prepared according to the method of Huntoon (1918), to which were added 3 drops of normal horse serum per 5 cc. of the medium. The strains of streptococci used were obtained from cases of empyema and were supplied by the Army Medical School. Hemolytic tests were accomplished by adding to 0.5 cc. amounts of the proper dilutions of the test substance 0.5 cc. of 5 per cent washed rabbit corpuscles. The tubes were then incubated for 1 hour in the water bath at 37°C. The relative susceptibility of the corpuscles from different animal species was not determined. It was found that the hemotoxin of the streptococcus was not specific for red blood cells but had the power of destroying various types of muscular and glandular tissue.

Effect of Filtration and Adsorption on the Hemotoxin.

The effect of filtration on hemotoxin may be determined by a few simple experiments. It was found that there was considerable loss during passage of bouillon through Mandler filters. If the hemolytic substance is held in the pores of the filter, reversal of the filter and washing with the filtrate should restore the hemolytic titer. No hemotoxin was recovered by this method. The rate of filtration was observed to have considerable effect upon the amount of hemotoxin in the filtrate; *i.e.*, the more rapidly the bouillon was forced through the filter, the greater the amount of hemotoxin recovered. As a control on this experiment a broken filter was finely pulverized in a mortar and 2 gm. were added to each of two tubes containing 5 cc. of hemolytic bouillon culture. These were shaken to insure an even distribution of the pulverized material throughout the bouillon. One was immediately centrifuged and the other allowed to stand for 30 minutes before centrifugation. The first tube showed slight diminution of hemolytic titer, while the second showed no hemotoxin present in the liquid.

TABLE I.

Treatment.	0.5 cc.	0.25 cc.	0.12 cc.	0.06 cc.	0.03 cc.	0.015 cc.
Untreated bouillon culture................	++	++	++	++	++	+
Rapid filtration........................	++	++	+	–	–	–
Slow "	–	–	–	–	–	–
Reversal of the filter.....................	–	–	–	–	–	–
Rapid adsorption........................	++	++	++	++	–	–
30 min. "	–	–	–	–	–	–
Addition of calcium chloride..............	+	–	–	–	–	–

++ designates complete hemolysis; +, partial hemolysis; –, no hemolysis.

The addition of calcium chloride to bouillon causes a precipitate which may act as an adsorptive agent. Barium carbonate will also remove the greater part of the hemotoxin from bouillon.

From Table I the similarity in action between rapid filtration and adsorption can be observed. It is especially significant, since the same material was used in both processes. The failure to recover hemotoxin by reversal of the filter and passage of the filtrate back through the filter indicates either the destruction of hemotoxin or its firm combination with the filter material. If infusion bouillon is passed through a diatomaceous filter before inoculation with hemolytic streptococci good growth results but the production of hemotoxin is greatly reduced, indicating that the basic substances from which the hemotoxin is derived are likewise adsorbed, at least in part, by filtration. The fact that the addition of insoluble compounds also removes hemotoxin from bouillon seems to confirm the part played by adsorption in the process of filtration.

Effect of Centrifugation and Shaking on the Hemotoxin.

If the hemotoxin is contained in or on the bacterial cells, concentration of the cells by centrifugation should increase the hemolytic titer. A tube of hemolytic bouillon was centrifuged at high speed until the supernatant liquid was clear (about 20 minutes). Another tube of bouillon fitted with a rubber stopper was placed on a wheel revolving about 30 times a minute and the bouillon was kept in constant agitation for 20 minutes. The top half of the liquid in the centrifuged tube was removed with a pipette; part of this was used for plating on blood agar in order to determine the number of cocci present and part used for hemolytic tests. The lower half was shaken in order to get an even distribution of the sedimented streptococci and was tested in a similar manner. As a control on the presence of streptococci some of the same bouillon was treated with the pulverized filter material for 20 minutes and centrifuged for 3 minutes. The results are shown in Table II.

The counts shown in this table are of necessity inaccurate, since we have no means of knowing whether one streptococcus or a chain of several members produced the colonies. They are, however, relative and give a fair ground for comparison. The hemotoxin has evidently disappeared from the supernatant fluid, although there are some organisms present. Even though the streptococci have been concentrated to twice the number per cubic centimeter the hemolytic titer is slightly less than that of the bouillon in the control tube which had not been centrifuged. The process of shaking also reduced the titer somewhat, although the bacterial count remained the same as in the control tube. The culture treated with kieselguhr contains enough streptococci to show some hemolysis, provided the hemolytic substance is an integral part of the cell. If a hemolytic test is set up in a hollow culture slide and the process of hemolysis studied through the microscope it soon becomes evident that hemotoxin must be free in the medium and unassociated with the bacterial cells. Red blood corpuscles are seen to assume gradually a moth-eaten appearance and finally to disintegrate, although during the whole process no streptococci have been near them. In such a test as that shown in Table II there are present 100 erythrocytes to each streptococcus. Since these organisms are non-motile it is difficult to believe that each blood cell has come in direct contact with a streptococcus. Microscopic study gives convincing evidence that such is not the case. The results of these experiments seem to indicate that the hemotoxin is not associated with the bacterial cells and that there is a mechanical factor responsible for loss of titer during centrifugation.

TABLE II.

Treatment.	0.5 cc.	0.25 cc.	0.12 cc.	0.06 cc.	0.03 cc.	0.015 cc.	No. of colonies per cc.
Untreated	++	++	++	+	–	–	1,000,000
Centrifuged, top	–	–	–	–	–	–	50,000
" bottom	++	++	+	+	–	–	∞
Shaken	++	++	+	–	–	–	1,000,000
Kieselguhr	–	–	–	–	–	–	100,000

Relation of Hydrogen Ion Concentration to the Hemotoxin.

Certain concentrations of acid and alkali are suitable reagents for causing the laking of erythrocytes. The possibility that hemolysis may be due to this cause cannot be ignored. The presence in bouillon of carbohydrates from which acid might be produced tends, however, to decrease rather than increase the amount of hemotoxin. The only apparent effect of the initial hydrogen ion reaction of the medium was to inhibit growth when the reaction was strongly acid or alkaline. Hemotoxin was obtained from bouillon having a pH value at the time of testing for hemolysis of 7.2 to 5.3. Media containing pieces of marble gave maximum hemotoxin production. Hemolytic bouillon diluted with an equal volume of isotonic sodium carbonate solution gave results identical with those obtained by diluting to the same extent with sodium chloride solution. From these results it seems impossible that the hemolytic action can be due to the presence of an acid radical.

Relation of the Constituents of the Medium to Hemotoxin Production.

The ideal method of attacking this phase of the problem would be to withdraw one substance after another from the culture medium and test each substance alone and also the remainder of the material as to its ability to support hemotoxin production. This is not possible in all cases on account of the complexity of the constituents of the bouillon. We must constantly bear in mind the necessity of removing any bactericidal reagent from the medium and also of retaining the tonicity of the preparations at a point such that hemolysis of red blood cells will not follow from this cause.

TABLE III.

Bouillon.	Growth.	0.5 cc.	0.25 cc.	0.12 cc.	0.06 cc.	0.03 cc.	0.015 cc.
Undiluted......................	Good.	++	++	++	++	++	+
Diluted to 2 volumes...........	“	++	++	++	++	++	+
“ “ 4 “	“	++	++	++	++	++	+
“ “ 8 “	“	++	++	++	++	+	+
“ “ 16 “	Slight.	++	++	++	+	−	−
“ “ 32 “	“	++	++	+	+	−	−
“ “ 64 “	“	++	+	+	−	−	−
Horse serum + salt solution......	None.	−	−	−	−	−	−

It seemed probable that on occasions solutions would be added to the medium and it would be inconvenient and not expedient to evaporate the excess. The effect of diluting the bouillon with physiological salt solution was therefore investigated. As usual, 3 drops of horse serum were added to each tube after the dilutions were made.

From Table III it is evident that bouillon can be diluted to four times its volume before any effect on hemotoxin production is observed. Beyond this point the hemotoxin production is in inverse ratio to the dilution of the medium. In no case has the addition of reagents approached four times the volume of the bouillon so any decrease in hemolytic titer is not due to the factor of dilution.

Relation of Phosphorus to Hemotoxin Production.—In Table I it was shown that the addition of calcium chloride to bouillon caused the formation of a precipitate. Upon analysis this proved to consist mainly of calcium phosphate, although other substances were brought down either mechanically or by adsorption. Phosphoproteins are not precipitated by this reagent. It was found, however, that streptococci during growth liberated phosphorus, presumably from the protein radicals. In order to obtain a phosphorus-free medium the following procedure was adopted. 0.6 cc. of 2.5 per cent calcium chloride solution was added to 5 cc. of bouillon and boiled. The precipitate was removed by centrifugation. The supernatant liquid was enriched with 3 drops of normal horse serum, inoculated with streptococci, and incubated for 24 hours. Then the process was repeated until the medium was free from phosphorus. Two or three inoculations were generally necessary. Such a phosphorus-free bouillon reinforced by 3 drops of normal horse serum supported abundant growth of streptococci but allowed production of no hemotoxin.

TABLE IV.

Bouillon.	Growth.	0.25 cc.	0.12 cc.	0.06 cc.	0.03 cc.	0.015 cc.
Bouillon B_1 with calcium chloride..........	Good.	++	++	++	++	+
" B_2 " " "	"	++	++	++	++	+
" B_3 " " "	"	–	–	–	–	–
" B_3 + ammonium monobasic phosphate..............................	"	++	+	+	–	–
Bouillon B_3 + phosphoric acid............	"	++	++	+	–	–
" B_3 + potassium acid phosphate....	"	++	–	–	–	–

The addition of known phosphorus compounds to a phosphorus-free bouillon was tried. In Table IV is a tabulation of a series of such experiments. Bouillon B_1 was heated with calcium chloride once; Bouillon B_2 was heated with calcium chloride, the precipitate removed, inoculated with streptococci, incubated, and precipitated a second time. No. B_3 was carried through the process of precipitation for a third time. When chemically tested this bouillon was shown to be free from phosphorus. The amounts of phosphorus compounds added were 0.5 cc. of 2 per cent ammonium monobasic phosphate, 0.5 cc. of 2 per cent potassium acid phosphate, and 0.5 cc. of 1 per cent phosphoric acid. These solutions are approximately isotonic for red cells as they gave no hemolysis when added to a standard cell suspension.

The results of these experiments indicate that as long as the phosphoproteins are present in the medium the maximum amount of hemotoxin is produced. The addition of phosphorus compounds restores in part but not completely the hemotoxin-producing properties of the medium. The non-production of hemotoxin in phosphorus-free bouillon is not due to the effect of the lack of buffer action, as the reaction was unchanged by the growth of the streptococci and presented a range within which hemotoxin has been produced repeatedly.

Relation of Other Chemical Factors to Hemotoxin Production.—The preceding experiment and certain others suggested the possibility that there might be present in the medium two factors or chemical essentials, one of which is able to support growth but not hemolysin production, the other supplies the necessary substances for the elaboration of hemotoxin. Experiments were instituted to separate the chemical substances contained in infusion bouillon. Metaproteins, proteoses, and peptone were isolated from the bouillon according to the methods outlined by Hawk (1916). These substances were made up to the approximate volume of the bouillon from which they were taken by the addition of physiological salt solution, and the standard amount of normal horse serum was added. Fair growth of streptococci was invariably obtained but no hemotoxin was produced. Even the mixture of all these substances in the original proportion failed to support the production of hemotoxin. It seems evident that a variety of substances may support growth but not the production of hemotoxin.

Two reagents have been used for the fractionating of bouillon, calcium chloride and ether. When calcium chloride is added to broth a heavy precipitate is formed. This precipitate can be divided by ether extraction into a waxy substance, which is perhaps cerebrin, and calcium phosphate. Neither the whole precipitate nor either of the fractions, when washed free of bouillon, supported hemotoxin production. Bouillon after removal of the calcium precipitate suffers no loss of its hemotoxin-producing qualities. Extraction of this fraction with ether does not affect the production of hemotoxin. When the whole bouillon is extracted with ether and then precipitated with calcium chloride the fractions containing the water-soluble matter are the only ones which give hemolysis. These preparations were brought as nearly as possible to the original volume and to them was added the usual amount of serum.

If the calcium precipitate previously referred to is returned to the bouillon no effect on hemotoxin production is observed. The substance separated from the bouillon by ether extraction, whether obtained from the whole bouillon or from the calcium precipitate, when returned to bouillon considerably reduces the yield of hemotoxin. Pure cholesterol when added to the bouillon does not influence hemotoxin production. Lecithin, however, has a pronounced inhibitory action. Thus the substance which is separated from the bouillon by means of ether acts in a manner similar to lecithin. The substances in bouillon which are soluble or insoluble in ether are not essential to the production of hemotoxin of the streptococcus.

Relation of Serum to Hemotoxin Production.—When bouillon prepared with Liebig's beef extract instead of meat infusion is planted with streptococci, fair growth is obtained but no hemotoxin is produced. By using this medium as a base, opportunity is offered for determining whether the source of the hemotoxin is in the infusion bouillon or in the blood serum. To one series of tubes containing 5 cc. of Liebig's extract bouillon graduated amounts of infusion bouillon were added; to the other the same amounts of serum were added. The results of these tests are shown in Table V.

From this table it can be seen that the infusion bouillon of itself adds but little to the production of hemotoxin, whereas serum increases the production in the ratio of its presence in the medium. There is, perhaps, the requisite amount of the growth factor in either Liebig's or infusion bouillon. The basic substances for hemotoxin production are present in only a slight degree in infusion bouillon but in considerable quantity in serum. It is evident that there are certain quantitative relations between the growth factor and the hemotoxin-producing factors to be observed in the production of the maximum amount of hemotoxin. With infusion bouillon 0.05 cc., or 3 drops, of serum are sufficient for maximum hemotoxin production, whereas with Liebig's extract bouillon 2 cc. of serum are necessary to obtain the same result. The growth factor in infusion bouillon may be diluted to four volumes as shown in Table III and still produce the maximum amount of hemotoxin. Horse serum diluted with physiological salt solution will support little if any growth.

The isolation of the hemotoxin-producing substance from serum is as difficult as from bouillon. Serum was diluted to twice its volume with salt solution and heated in boiling water until the coagulable proteins were well separated. This preparation was centrifuged until the supernatant fluid, or serum water as we have designated it, was perfectly clear. When this serum water which amounted to 5 to 10 per cent of the original serum was made up to volume and added to bouillon, hemotoxin was produced in maximum quantity. Calcium chloride solution, when added to serum water, causes a precipitate, but the hemotoxin-producing factor is but slightly diminished in the supernatant fluid. The coagulable protein and the calcium precipitate of serum water do not support hemotoxin production and growth occurs to only a slight degree.

TABLE V.

Amount of infusion bouillon + 5 cc. of Liebig's extract bouillon.	Hemolysis produced.					Amount of horse serum + 5 cc. of Liebig's extract bouillon.	Hemolysis produced.				
	0.25 cc.	0.12 cc.	0.06 cc.	0.03 cc.	0.015 cc.		0.25 cc.	0.12 cc.	0.06 cc.	0.03 cc.	0.015 cc.
cc.						*cc.*					
0.05	+	–	–	–	–	0.05	–	–	–	–	–
0.2	++	+	–	–	–	0.2	+	–	–	–	–
0.6	++	+	–	–	–	0.6	++	++	+	+	–
1.0	++	+	–	–	–	1.0	++	++	++	+	–
2.0	++	+	–	–	–	2.0	++	++	++	++	++
2 cc. of infusion + 5 cc. of salt solution.	++	++	++	++	–	2 cc. of horse serum + 5 cc. of salt solution.	–	–	–	–	–

Hemotoxin-Producing Substance in Various Organs.—The occurrence of the hemotoxin-producing substance in different organs was investigated. Water extracts of brain, liver, and kidney were prepared by infusing the macerated tissues in an equal weight of water. The meat and precipitate were removed by centrifugation and the clear extract was sterilized by boiling for 5 minutes. These preparations were added to Liebig's extract bouillon in amounts of 0.6 cc., a quantity in the case of serum sufficient to produce a fair amount of hemotoxin. The brain extract induced little or no hemotoxin production due, evidently, to the presence of a lipoid in the medium. The liver extract produced an unusual amount of growth but no hemotoxin. This result we considered as due to the selective action of the streptococcus for the glycogen in the liver extract. The kidney extract, however, when added to Liebig's bouillon produced hemotoxin equal in amount to that elaborated in the presence of serum or serum water. These results are similar to those obtained by Kligler (1919) in his study of the growth-producing substances in tissue.

The outstanding feature of these substances is their susceptibility to alkalinity and high temperatures. Kidney infusion was distributed in 5 cc. amounts into four test-tubes. To the first tube 1 cc. of normal potassium hydroxide was added; it was boiled for 5 minutes and then exactly neutralized with normal hydrochloric acid. To the second tube were added 1 cc. of normal potassium hydroxide and 1 cc. of normal hydrochloric acid and the whole was boiled at the same time as the first. To the third tube were added 2 cc. of sodium chloride solution and the entire contents were boiled as a control when the other tubes were boiled. The fourth tube was autoclaved for 30 minutes at 15 pounds pressure. The contents of the first tube when added to Liebig's extract bouillon in any amount failed to support hemotoxin production. The contents of the second and third tubes induced hemotoxin production equal to that obtained with serum. The autoclaved infusion acted in a manner comparable to that of infusion bouillon as shown in Table V.

At first one is tempted to designate the hemotoxin-producing substance by the term vitamine, as did Kligler (1919). It is doubtful whether the word as used by the physiological chemists can be justified in this application in bacteriology. The distinction between the growth factor and the factor producing hemotoxin is marked. A variety of substances may supply the needs for metabolism. Proteoses, peptone, probably some of the amino-acids, and sugars furnish the essentials for growth. The substance essential for hemotoxin production does not support the growth of the organisms. We believe that hemotoxin is a compound synthesized from at least two basic substances; one of these is a phosphorus compound, the other a substance of unknown composition. The latter is present in fresh tissue and body fluids, is water-soluble, and is susceptible to certain destructive processes. The hemotoxin compound is easily destroyed by mechanical and physical methods and acts as an organic compound and not as an enzyme.

CONCLUSION.

The hemotoxin of streptococcus is a labile substance affected by centrifugation or shaking. It is adsorbed by various organic and inorganic substances. Hemotoxin is produced within a wide range of hydrogen ion concentrations. It is neither in nor on the bacterial cell but is free in the culture medium. It is probably not an enzyme. There are at least two substances which are essential to the medium for the elaboration of hemotoxin, one of which is phosphorus; the other is a substance of unknown composition. The unknown component is present in small quantities in unfiltered muscle infusion, but is more abundantly supplied by blood serum and kidney infusion. This substance is not an albumin, globulin, primary or secondary proteose, metaprotein, or peptone of the medium or enriching fluid. It is water-soluble, is destroyed by boiling in alkaline solution and by prolonged heating, and is removed to a considerable extent by passage through a diatomaceous filter.

BIBLIOGRAPHY.

Aronson, H., *Berl. klin. Woch.*, 1902, xxxix, 979.

Besredka, *Ann. Inst. Pasteur*, 1901, xv, 880.

Braun, H., *Centr. Bakt.*, *1te Abt.*, *Orig.*, 1912, lxii, 383.

Hawk, P. B., Practical physiological chemistry, Philadelphia, 5th edition, 1916.

von Hellens, O., *Centr. Bakt.*, *1te Abt.*, *Orig.*, 1913, lxviii, 602.

Huntoon, F. M., *J. Infect. Dis.*, 1918, xxiii, 169.

Kligler, I. J., *J. Exp. Med.*, 1919, xxx, 31.

Levin, E., *Nord. med. Arch.*, 1903, iii, 3 F., 1.

von Lingelsheim, W., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1912, iv, 453.

Lyall, H. W., *J. Med. Research*, 1914, xxx, 487.

M'Leod, J. W., *J. Path. and Bact.*, 1911–12, xvi, 321.

Nakayama, Y., *J. Infect. Dis.*, 1919, xxv, 509.

Ruediger, G. F., *J. Am. Med. Assn.*, 1903, xli, 962.

Schlesinger, A., *Z. Hyg. u. Infectionskrankh.*, 1903, xliv, 428.

A COMPARATIVE STUDY OF HEMOLYTIC STREPTOCOCCI FROM MILK AND FROM HUMAN LESIONS.

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A COMPARATIVE STUDY OF HEMOLYTIC STREPTOCOCCI FROM MILK AND FROM HUMAN LESIONS.*

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The work presented in this paper was undertaken primarily to study the characters which differentiate hemolytic streptococci which are pathogenic for man from non-pathogenic hemolytic streptococci occurring in milk. Several papers in which the finding of hemolytic streptococci in milk is reported have appeared within the last few years. These streptococci are generally considered to be non-pathogenic, except in a few cases, in which hemolytic streptococci have been found in milk during epidemics of septic sore throat, and were thought to be the etiological agent in the disease.

Streptococci which produce a clear zone around the colony on a blood agar plate are usually regarded as hemolytic. There are wide variations among streptococci in their ability to produce zones of hemolysis. In fact, most streptococci and some pneumococci cause a slight clearing of blood agar around the colony. Smith and Brown (1) have studied the hemolytic zones made by streptococci on standardized blood agar plates, and have classified them according to the appearance of the zone of hemolysis. It was found that hemolytic streptococci of the typical human pathogenic type gave a perfectly well-defined zone of hemolysis, two to four millimeters in diameter. These streptococci were recognizable on blood agar plates, and were found to be a group with definite pathogenic properties. This group is designated by Smith and Brown as the Beta type.

It has been observed by several investigators, however, that streptococci which seem to have no pathogenic properties, but which produce, on blood agar plates, zones of hemolysis closely resembling the Beta type, frequently occur in milk. Davis (2) found streptococci of this kind in both raw and pasteurized milk, but more commonly in the former. Most of them were non-virulent for rabbits. Brown (3), in a recent paper, points out some of the characteristics which differentiate milk strains from human pathogenic strains.

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Preliminary examination of pasteurized milk in Baltimore showed that streptococci of the Beta type of hemolysis were present in the milk, in some cases in large numbers. A survey was made of the milk of a number of dairies to determine how generally these streptococci occurred. The bottled milk was bought from the dealers and plated on standard blood agar plates. Plain agar was used in the preparation of the blood agar. This was tubed in 12 c.c. portions, and to the melted and cooled agar 0.5 c.c. of whole rabbit's blood was added. Dilutions of the milk were made and plates were poured. The plates were incubated forty-eight hours at 37° C. The number of colonies showing hemolysis of the Beta type were counted and recorded. The numbers of these hemolytic streptococci in the milk of a given dairy varied widely from day to day. Also the proportion of hemolytic streptococci to other organisms varied widely. Table I represents typical counts of the milk of several dairies.

TABLE I.

The occurrence of hemolytic streptococci in milk.

Dairy.	Bacteria per c.c.	Hemolytic streptococci per c.c.	Per cent. of hemolytic streptococci.
A..........	45 000	3 600	8.0
B..........	22 000	320	1.4
C..........	157 000	340	0.2
D..........	28 000	None	0.0
*E..........	8 300	530	6.4
F..........	56 000	525	0.9
G..........	42 500	100	0.9
B..........	15 000	190	1.3
B..........	12 200	123	1.0
H..........	44 500	8 500	19.1
*E..........	6 950	225	3.2
D..........	26 000	None	0.0
B..........	22 500	100	0.4
I..........	55 600	None	0.0

The similarity of these pathogenic and non-pathogenic streptococci, on blood agar plates, and the frequency with which the latter occur in milk render the study of characteristics which differentiate them of importance.

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* Certified milk.

METHODS.

From a study of the literature on differentiation and classification of streptococci it becomes apparent that no single character will suffice to distinguish a group, though by the use of a group of characters it may be possible to define a fairly homogeneous group. In this work a study of the hemolytic streptococci which occur in milk was made with a threefold purpose in mind. It was purposed, first, to learn the characteristics of these streptococci and to see whether or not they constitute a homogeneous group; second, to determine the characters which are most useful in differentiating them from hemolytic streptococci which are pathogenic for man; and third, to study the thermal death rate of this group and of other hemolytic streptococci in milk. The following characters were selected for study:

1. Morphology.
2. Fermentation reactions.
3. Hemolysis.
4. Pathogenicity for mice.
5. Reduction of litmus and coagulation of milk.
6. Thermal death rate, in milk, at 60° C.

The milk strains used in this study were isolated from milk by plating on blood agar. Most of them were taken from bottled pasteurized milk, but some were from certified, unpasteurized milk. The blood agar was prepared by adding citrated whole rabbit blood to plain agar. One half c.c. of blood was added to each tube containing 12 c.c. of the melted and cooled medium. The dilutions of milk were made and then mixed with the agar on the plate. The plates were incubated 24 hours at 37° C.

Only those colonies which gave wide, clear, well-defined zones of hemolysis were selected. Colonies showing every degree of hemolysis from a barely perceptible halo to the wide, clear zones were found on most of the plates. Some colonies gave wide zones, but the outer limits of the zones were not well defined. Such colonies, when fished to broth and the broth tested with washed red cells gave no hemolysis. Only those which had the wide, clear-cut zones were found to respond to this test. These colonies correspond to Smith and Brown's Beta type. After a little experience it was found possible to pick these colonies with considerable accuracy.

The selected colonies were transferred to veal infusion broth. Smears were made from the broth cultures and examined for purity.

Stock blood agar slants were made from the pure broth culture. All tests were made upon transfers from these stock cultures.

For comparison with the milk strains a number of cultures from pathological sources were selected. Most of these were obtained from the Hygienic Laboratory of the United States Public Health Service, Washington, D. C. These cultures had been isolated mainly from empyema cases in army camps. Others were obtained from other sources, as shown in Table III.

TABLE II.

Milk cultures.

Culture.	Source.	Morphology.	
		Cells.	Predominating groups.
B 1...	Standard Pasteurized Milk	Oval	Short chains and pairs
B 2...	Standard Pasteurized Milk	Oval	Short chains and pairs
B 3...	Standard Pasteurized Milk	Oval	Long chains, cells paired
B 4...	Standard Pasteurized Milk	Oval	Short chains and pairs
B 5...	Standard Pasteurized Milk	Oval	Long chains, cells paired
F 4...	Standard Pasteurized Milk	Oval	Short chains and pairs
F 7...	Standard Pasteurized Milk	Oval	Short chains and pairs
F 8...	Standard Pasteurized Milk	Oval	Short chains and pairs
F 9...	Standard Pasteurized Milk	Oval	Short chains and pairs
F10...	Standard Pasteurized Milk	Oval	Short chains and pairs
F11...	Standard Pasteurized Milk	Oval	Pairs, a few short chains
F12...	Standard Pasteurized Milk	Oval	Pairs, a few short chains
F13...	Standard Pasteurized Milk	Oval	Pairs, a few short chains
F14...	Standard Pasteurized Milk	Oval	Pairs, a few short chains
C 4...	Standard Pasteurized Milk	Large oval	Pairs and short chains
C 7...	Standard Pasteurized Milk	Large oval	Pairs and short chains
C 8...	Standard Pasteurized Milk	Oval	Long chains
C 9...	Standard Pasteurized Milk	Oval	Short chains and pairs
C10...	Standard Pasteurized Milk	Oval	Short chains and pairs
W 1...	Certified Milk	Spherical	Long chains
W 2...	Certified Milk	Spherical	Long chains
W 3...	Certified Milk	Spherical	Long chains
W 4...	Certified Milk	Spherical	Short chains
W 5...	Certified Milk	Spherical	Short chains
W 6...	Certified Milk	Spherical	Short chains and pairs
W 8...	Certified Milk	Large oval	Long chains
W 9...	Certified Milk	Spherical	Short chains
D 6...	Standard Pasteurized Milk	Large oval	Short chains and pairs
D 7...	Standard Pasteurized Milk	Large oval	Short chains and pairs
D 8...	Standard Pasteurized Milk	Large oval	Short chains and pairs
D 9...	Standard Pasteurized Milk	Large oval	Short chains and pairs
D10...	Standard Pasteurized Milk	Large oval	Short chains and pairs
D11...	Standard Pasteurized Milk	Spherical	Short chains and pairs
D12...	Standard Pasteurized Milk	Oval	Short chains and pairs
U 1...	Standard Pasteurized Milk	Oval	Short chains and pairs
U 2...	Standard Pasteurized Milk	Oval	Short chains and pairs
U 3...	Standard Pasteurized Milk	Oval	Long chains, cells paired
U 4...	Standard Pasteurized Milk	Oval	Short chains and pairs

MORPHOLOGY.

A study of the morphology of the milk strains was made soon after isolation. For this purpose they were grown in meat infusion broth, and stained by Gram's method, after 24 hours growth. The human strains were grown in broth and examined in the same way. Tables II and III show the summary of the points observed. Examination was also made of the growth from blood agar and from milk. In general it may be said that the organisms were more uniform in size and shape when grown in milk than when grown in other media. In broth, and especially on blood agar, the organisms tended to develop irregular shapes and sizes. This was more marked in the milk strains than in the human strains.

TABLE III.

Human pathogenic cultures.

Culture.	Source.	Morphology.	
		Cells.	Predominating groups.
24	Middle ear infection	Spherical	Long chains
177	Puerperal septicemia	Spherical	Long chains
179	Cerebrospinal meningitis	Spherical	Long chains
180	Scarlet fever	Spherical	Long chains
182	Septicemia	Oval	Long chains
235	Empyema	Spherical	Long chains
234	Empyema	Spherical	Long chains
238	Empyema	Spherical	Long chains, cells paired
240	Empyema	Spherical	Long chains
242	Empyema	Spherical	Long chains
250	Empyema	Spherical	Long chains
252	Empyema	Spherical	Long chains, cells paired
259	Empyema	Oval	Long chains, cells paired
260	Empyema	Spherical	Long chains, cells paired
261	Empyema	Spherical	Very long chains
200	Empyema	Large spherical	Long chains
247	Empyema	Spherical	Long chains
258	Empyema	Oval	Long chains and pairs
7	Septicemia	Spherical	Long chains
44	Septicemia	Spherical	Long chains
33	Empyema	Spherical	Long chains, cells paired
18	Septicemia	Spherical	Long chains and pairs
DB	Empyema	Spherical	Short chains
Viridans			
186	Pyorrhea	Spherical	Short chains
LI	Tonsilitis	Spherical	Short chains

General morphology and chain formation varied considerably among the various cultures of both groups. All cultures showed chain formation, but the proportion of the whole number of organisms in chains, and the number of elements in the chains were points of variation. In some of the milk strains pairs or diplococci predominated, while in others practically all of the organisms were in chains of several elements. The human strains were not so variable in this respect. Most of them were in chains of varying lengths. Variations in the size and shape of the cells were also more marked in the milk strains. Some showed small and delicately spherical cells, while others were larger and somewhat elongated.

The outstanding morphological differences between the organisms of the two groups may be summed up as follows:

Milk strains:

Cells elongated-oval, varying greatly in size; organisms less regularly in long chains, and whether in chains or not, the arrangement of cells was quite uniformly in pairs.

Human pathogenic strains:

Cells spherical, more regular in size and shape; chains usually long and few organisms not in chains; some, but not all, showed a tendency to diplococcus form.

Some cultures in each group are indistinguishable morphologically from those of the other group, but the majority of each showed the differences outlined. These characters remained constant, when observed in the same medium, after months of cultivation.

The morphology alone is thus of little value in distinguishing bovine from human strains but, when used with other characters, it will aid in the diagnosis of an unknown culture.

THE REACTION IN LITMUS MILK.

In the study of the reaction of streptococci in litmus milk, two distinct characters are involved, namely, the coagulation of the milk and the reduction of the litmus. Both have been found of some differential value by various observers. Observations which have been made on these characters are mentioned elsewhere. In addition it has been observed by several investigators, viz., Esten (4), Sherman (5), and Evans (6), that the behavior of streptococci of the lacticus type, in litmus milk, is characteristic. This characteristic reaction is used in some laboratories of dairy bacteriology to identify members of this group. The typical streptococcus lacticus, according to Evans, coagulates milk within 24 to 48 hours. This coagulation is accompanied by a reduction of the litmus, beginning at the bottom of the tube and progressing nearly to the surface, leaving a pure white column of milk with a pink layer on top.

All the cultures of human and milk strains of hemolytic streptococci were inoculated into litmus milk. The tubes were filled to a uniform depth, of about one and one-half inches, and the litmus in all tubes was as nearly as possible of equal concentration. The inoculated tubes were incubated at 37° C. and observed at frequent intervals. Record was made of the reaction after 24 hours and after 48 hours. The results are shown in Tables IV and V.

TABLE IV.

Reactions of pathogenic cultures in litmus milk.

Culture.	Reaction After.	
	24 Hours.	46 Hours.
24	No change	A
177	No change	A
179	No change	A
180	No change	No change
182	No change	No change
200	No change	A
233	No change	A
236	No change	A
240	No change	A
238	No change	No change
242	No change	A
247	A	A C
250	No change	No change
252	No change	No change
258	No change	A
259	No change	A
260	No change	A
261	No change	A
33	A	A
44	No change	No change
7	No change	No change
18	A	A C
106 (vir.)	R A C	R A C
DB	R A	R A C

R = Reduction of litmus.
A = Acid.
C = Coagulation.

TABLE V.

Reactions of milk cultures in litmus milk.

Culture.	Reaction After. 24 Hours.	Reaction After. 48 Hours.
B 1	R A	R A C
B 2	R A	R A C
B 3	R A	R A C
B 4	R A	R A C
B 5	R A	R A C
F 6	R A	R A C
F 7	R A C	R A C
F 8	R A	R A C
F 9	R A	R A C
F 10	R A C	R A C
F 11	R A C	R A C
F 12	R A C	R A C
F 13	R A C	R A C
F 14	R A C	R A C
C 4	R A	R A C
C 7	R A	R A C
C 8	R A	R A C
C 9	R A	R A C
C 10	R A	R A C
W 1	No change	A
W 2	No change	A
W 3	No change	A
W 4	No change	A
W 5	No change	A
W 6	No change	A
W 8	No change	A
W 9	No change	A
D 6	R A C	R A C
D 7	R A C	R A C
D 8	R A C	R A C
D 9	R A C	R A C
D 10	R A C	R A C
D 11	R A C	R A C
D 12	R A C	R A C
U 1	R A C	R A C
U 2	R A C	R A C
U 3	R A C	R A C
U 4	R A C	R A C

R = Reduction of litmus.
A = Acid.
C = Coagulation.

It will be seen that the majority of the milk strains produce acid and cause a reduction of the litmus in 24 hours. Some cause coagulation of the milk also, but in most cases this occurred between the 24 and the 48 hour readings. Reduction of the litmus in the bottom of the tube appeared, in most cases, before any color change took place and by the time sufficient acid was formed to cause a pink coloration, only a layer about ⅛ to ¼ inch thick at the top remained colored, the rest being pure white. This shallow layer persisted for about three days and then deepened slowly, until at the end of a week about half of the column of milk was pink. The hemolytic streptococci from milk, therefore, give the same litmus milk reaction as do the members of the *S. lacticus* group.

Not all of the milk strains give this typical reaction, however. A small number acidify the milk very slowly, and cause no coagulation or reduction of litmus. Special mention should be made of the "W" strains. These are from a high grade of certified milk. This milk is produced under conditions which would, as nearly as possible by ordinary precautions, exclude organisms from such sources as the cow's flanks, the hand of the milker, the stable air, and unsterilized utensils. The organisms present would therefore be largely represented by those coming from the interior of the udder. Sherman and Albus (5) considered the streptococci obtained from the udder of the cow to be of the pyogenes type, and found them distinctly different, in a number of characters, from the lactic acid type. It is possible, therefore, that these milk strains which do not coagulate milk and reduce litmus are of the pyogenes type. The subsequent tests support this view.

The human pathogenic strains act slowly upon litmus milk. Little change is seen in 24 hours. Many of them show a weak acidity in 48 hours, while others cause little or no apparent change in the milk, even after several days incubation. Reduction of litmus was brought about by but one strain; two strains caused coagulation. Two strains of *S. viridans* which were included for comparison caused both reduction and coagulation.

In a general way, hemolytic streptococci from milk, produced under ordinary conditions, bring about a rapid acidification, coagulation, and reduction in litmus milk, while human pathogenic strains only produce a slight acidity. The exceptions, however, are too numerous to use this test for absolute differentiation.

Fermentation of carboyhydrates.

The fermentation tests were made in a veal infusion broth which was prepared after the method described by Huntoon, that is, the broth was not filtered through cotton or filter paper, but was allowed to clarify by sedimentation in tall jars. This medium gave abundant growth of all strains without the addition of carbohydrates. In preliminary tests the broth was inoculated with *B. coli* to rid it of muscle sugar, but by comparison with controls this proved to be unnecessary and was abandoned. In all tests, however, control tubes to which no carbohydrate was added were inoculated and tested with the rest.

The test substances were made up in 10 per cent. solutions and sterilized separately. One per cent. of the test substance was added to the tubed and sterilized broth. The tubes were then incubated for 24 hours to insure sterility. Transfers were made to plain infusion broth from the stock cultures of the organisms to be tested. After 24 hours incubation 0.1 c.c. of this culture was added to each of the test broth tubes. All tubes were incubated for seven days at 37° C., including the tube from which seeding was made, this tube being the control mentioned above. Two drops of Andrade's indicator were added to each tube, at the end of the incubation period, and the tube was allowed to stand for a few minutes before being read. Little difficulty was encountered with doubtful reactions, nearly all being distinctly positive or negative. None of the controls showed any color with the indicator.

A tabular view of the results may be seen in Tables VI and VII. The only remarkable feature of these results is the uniformity with which saccharose is fermented by the human pathogenic strains, and the negative results obtained with this sugar when tested with the milk strains. The exceptions are nearly all in the "W" strains which resemble the human strains in their fermentation reactions. It was noted in the preceding test that they also reacted in litmus milk, as do the human pathogens.

These results are similar to those obtained by Sherman and Albus (5), who found that pyogenic streptococci fermented saccharose, while streptococci of the lacticus type did not. Their pyogenic cultures were isolated from the udder of cows, and were probably like the "W" strains in this table.

In the several classifications of streptococci, based on fermenta-

TABLE VI.

Fermentation of carbohydrates by milk cultures.

Culture.	Dextrose.	Lactose.	Saccharose.	Raffinose.	Salicin.	Inulin.	Mannite.	Control.
U 1	+	+	−	−	+	−	−	−
U 2	+	+	−	−	+	−	−	−
U 3	+	+	−	−	+	−	−	−
U 4	+	+	−	−	+	−	−	−
C 7	+	+	−	−	+	−	−	−
C 8	+	+	−	−	+	−	−	−
C 9	+	+	−	−	+	−	−	−
C10	+	+	−	−	+	−	−	−
F 7	+	+	−	−	+	−	−	−
F 8	+	+	−	−	+	−	−	−
F 9	+	+	−	−	+	−	−	−
F10	+	+	−	−	+	−	−	−
F11	+	+	+	−	+	−	−	−
F12	+	+	−	−	+	−	−	−
F13	+	+	+	−	+	−	−	−
F14	+	+	+	−	+	−	−	−
W 1	+	+	+	−	+	−	+	−
W 2	+	+	+	−	+	−	−	−
W 3	+	+	+	−	+	−	−	−
W 4	+	+	+	−	+	−	+	−
W 5	+	+	+	−	+	−	+	−
W 6	+	+	+	−	+	−	+	−
W 8	+	+	+	−	+	−	−	−
W 9	+	+	+	−	+	−	+	−
D 7	+	+	−	−	+	−	−	−
D 8	+	+	−	−	+	−	−	−
D 9	+	+	−	−	+	−	−	−
D10	+	+	−	−	+	−	−	−
D11	+	+	−	−	+	−	−	−
D12	+	+	−	−	+	−	−	−

tion, this saccharose-negative, hemolytic streptococcus seems to have been overlooked. Andrews and Horder (7) have no saccharose negative groups, and Holman (8) does not use saccharose in his classification. In the latter classification, lactose and salicin-positive, mannite-negative, hemolytic streptococci, such as are shown in the table, would fall into the S. pyogenes group. So also would the human pathogenic strains and these two groups would not be differentiated.

It appears from the literature that the majority of milk streptococci ferment saccharose. *Streptococcus lacticus* is so descirbed in textbooks. Savage (9) stated that most milk streptococci ferment saccharose. Houston (10) found this true of 90 per cent. of milk streptococci, while Broadhurst (11) found only 66 per cent. of milk strains saccharose fermenters.

Sherman and Albus (5), on the other hand, believe that typical *Streptococcus lacticus* does not ferment saccharose and their experiments seem to show that the fermentation of this sugar distinguishes *S. pyogenes* of cows' udders from *S. lacticus.*

TABLE VII.

Fermentation of carbohydrates by human pathogenic cultures.

Culture.	Dextrose.	Lactose.	Saccharose.	Raffinose.	Salicin.	Inulin.	Mannite.	Control.
24	+	+	+	−	+	−	−	−
177	+	+	+	−	+	−	−	−
179	+	+	+	−	+	−	−	−
180	+	+	+	−	+	−	+	−
182	+	−	+	−	+	−	+	−
200	+	+	+	−	+	−	−	−
233	+	+	+	−	+	−	−	−
234	+	+	+	−	+	−	−	−
238	+	+	+	−	+	−	−	−
240	+	+	+	−	+	−	−	−
247	+	+	+	−	+	−	−	−
242	+	+	+	−	+	−	−	−
250	+	+	+	−	+	−	+	−
252	+	+	+	−	+	−	−	−
258	+	+	+	−	+	−	−	−
259	+	+	+	−	+	−	−	−
260	+	+	+	−	+	−	−	−
261	+	+	+	−	+	−	−	−
44	+	+	+	−	+	−	+	−
33	+	+	+	−	+	−	−	−
7	+	+	+	−	+	−	−	−
DB	+	+	+	+	+	+	−	−
LI (vir.)	+	+	+	+	+	−	−	−
166(vir.)	+	+	+	+	+	+	−	−

From the data, presented in Tables VI and VII, it appears that saccharose may be useful in differentiating non-pathogenic hemolytic streptococci of milk from those of human origin.

Hemolysis.

As stated before, all of the strains used in this study were isolated by picking typical hemolytic colonies from blood agar plates. In so doing only those colonies were selected which gave the Beta type of hemolysis (Smith and Brown). In the appearance on blood agar plates, therefore, all of the milk strains were practically identical, and they proved to be identical in appearance with the human pathogenic strains when grown on blood agar plates prepared in the same manner. The two groups were therefore indistinguishable by blood agar plate hemolysis.

All cultures were then further tested for the production of a soluble hemolysin in broth, as recommended by the medical department of the United States Army in a leaflet entitled, "The Isolation and Identification of Streptococcus Hemolyticus." The cultures were grown in infusion broth for 18 to 24 hours. One-half c.c. of broth culture was then added to one-half c.c. of a 5 per cent. saline suspension of washed rabbit red blood cells and incubated for two hours. Positive controls of known hemolytic cultures and negative controls with sterile broth were included in each test. Only those cultures which gave complete hemolysis in two hours were retained for study. All of the colonies which gave a wide, absorbing clear zone with well-defined limits responded to this test. Occasionally colonies were picked which gave wide, clear zones of hemolysis, but which, on close inspection showed some red color remaining in the zone. These cultures always gave negative results in broth. Others again had wide, clear zones but the outer border was not sharply defined. These also gave negative results in broth.

After some study, one may predict with considerable accuracy the reaction in broth by the appearance of the zone of hemolysis on a standard blood agar plate. The test for hemolysin in broth culture, therefore, seems to correlate with a definite appearance on blood agar plates and separates a group of hemolytic streptococci with definite hemolytic properties. It gives clear-cut results, that is, distinctly positive or negative.

It seems preferable to use this test to confirm results obtained with the blood agar plate. Variations in technique and in the personal judgment of different investigators render the reports of types of hemolysis on blood agar plates too uncertain. If a culture is reported as positive or negative in the broth test, its hemolytic properties are more clearly defined. Swabs were taken from ten normal throats of a class in bacteriology. All showed hemolytic colonies in varying numbers. The zones of hemolysis were wide, with well-defined borders. They were hardly distinguishable from the true Beta type. None of the ten, however, gave positive results in broth cultures. This suggests that perhaps the widely varying results reported in regard to the occurrence of hemolytic streptococcus in normal throats may be due to variations in what are considered to be true hemolytic streptococci.

It is evident that neither of the hemolysis tests mentioned suffices to distinguish Beta hemolytic streptococci from milk and from human lesions. Brown, in a recent paper, noted that cultures of milk strains

TABLE VIII.

Rate of hemolysis with human and bovine strains.

(Hemolysis after thirty minutes.)

Milk culture.	Dilution.					Human culture.	Dilution.				
	1/2	1/4	1/8	1/16	1/32		1/2	1/4	1/8	1/16	1/32
U 1...	++	+				24...	++++	+++	+++	++	+
U 2...	++	+				177...	++++	++++	+++	++	+
U 3...	+					179...	++++	++++	+++	+++	
W 1...	++++	++++	+++			180...	++++	++++	++++	++++	+++
F 7...	++	+				182...	++++	++++	++++	++++	+++
F 8...	+					233...	++++	+++	+++	++	
F 9...	+	+				234...	++++	+++	+++	+	
F10...	++					238...	+++	+++	+++	++	+
C 8...	++					240...	++++	++++	+++	++	+
C10...	++					242...	++++	++++	+++	++	
D 6...	+					33...	++++	++++	++++	++++	+++
D 7...	++	+				259...	++++	++++	++++	++++	+++
D 8...	++++	+++	+			260...	++++	++++	++++	++++	+++
D12...	++	+				261...	++++	++++	++++	+++	++
W 2...	+++	++				252...	++++	++++	++++	++++	++
W 3...	++	+				258...	++++	++++	++++	+++	++
B 3...	+++	++	+			247...	++++	++++	++++	+++	++
B 4...	++	+				250...	++++	++++	++++	++++	+++
B 5...	++	+				DB...	++	++	+		
B 1...	+					7...	++	++	+		
D10...	+					41...	++++	++++	++++	++	+
D 9...	+										
W 8...	++++	++									
W 6...	++++	++++	++++	++++	++++						
W 3...	++++	++++	++++	++++	+++						
D11...	+++	++	+								
F12...	++	+									
F11...	+	+									

TABLE IX.

Rate of hemolysis with human and bovine strains.

(Hemolysis after ninety minutes.)

Milk culture.	Dilution.					Human culture.	Dilution.				
	1/2	1/4	1/8	1/16	1/32		1/2	1/4	1/8	1/16	1/32
U 1	++++	++++	+++	++	+	24...	++++	++++	++++	++	+
U 2..	++++	++++	++++	+++	++	177...	++++	++++	++++	++++	++
U 3	++++	++++	++++	+++		179...	++++	++++	++++	++++	+++
W 1	++++	++++	++++	++++	++++	180...	++++	++++	++++	+++	++
F 7	++++	++++	++++	++++	++	182...	++++	++++	++++	++++	++++
F 8	++++	++++	++++	++		233...	++++	++++	++++	+++	++
F 9	++++	++++	++++	++		234...	++++	++++	++++	+++	++
F10	++++	++++	++++	++		238...	++++	++++	++++	++	
C 8.	++++	++++	++++	++		240...	++++	++++	++++	++	++
C10..	++++	++++	++++	++		242...	++++	++++	++++	++	+
D 6 ..	++++	++++	++++	++++	++++	33...	++++	++++	++++	++++	++++
D 7...	++++	++++	++++	++++	++++	259...	++++	++++	++++	++++	+++
D 8...	++++	++++	++++	++++	++++	260...	++++	++++	++++	++++	++++
D12...	++++	++++	++++	++++	++++	261...	++++	++++	++++	++++	+++
W 2...	++++	++++	++			252...	++++	++++	++++	++++	++++
W 3...	++	++				258...	++++	++++	++++	++++	++
B 3...	++++	++++	++++	++++	++++	247...	++++	++++	++++	++++	++
B 4...	++++	++++	++++	++++	++++	250...	++++	++++	++++	++++	++
B 5...	++++	++++	++++	++++	++++	DB...	++++	++++	++		
B 1...	++++	++++	++++	++++	++++	7...	++++	+++	+		
D10...	++++	++++	++++	++++	++++	44...	++++	++++	++++	++++	++
D 9...	++++	++++	++++	++++	++						
W 8...	++++	++++	++++	++	+						
W 6...	++++	++++	++++	++++	++++						
W 5...	++++	++++	++++	++++	+++						
D11..	++++	++++	++++	++++	++++						
F12...	++++	++++	++++	++++	+++						
F11 .	++++	++++	++++	++++	+++						

of the Beta type require a longer period of incubation with the red cell suspension to produce hemolysis. This fact had often been observed in the present work, and an experiment was planned to compare the two groups of cultures in this respect, and also to compare their hemolytic titer. Tables VIII and IX show the results of the test. The cultures were diluted with saline, incubated at the same time in a 37° C. water bath, and the results read at the end of 30 and 90 minutes. It will be seen that the milk strains have a very low titer after 30 minutes, while with the human strains hemolysis is nearly complete. The one "W" strain included resembles the human strains as in previous tests. After 90 minutes there is no striking difference in the hemolytic titer of the two groups. These results agree with those of Brown (3), and it is evident that the rate of lysis of blood cells is more rapid with human strains.

Another observation in connection with tube hemolysis of the milk strains should be noted, namely, the production of brownish discoloration of the cells before they are laked. This occurred with all of the milk strains, although it was more pronounced in some than in others. The color remained and became intensified after the lysis of the cells. Occasionally some of the human strains showed a slight browning of the hemoglobin. Spectroscopic examination of the brown colored tubes showed the characteristic absorption bands of methemoglobin in the red, yellow, and green.

Pathogenicity for mice.

Streptococci vary so greatly in their pathogenicity for animals that the value of inoculation experiments may easily be overestimated. It is very difficult to get constant results even with cultures of known pathogenicity. Thus mouse protection tests of antistreptococcic serums have not proven of great significance in judging the value of the serum, because of the inconstancy of control inoculations. The inoculation of animals with a few strains of a group of streptococci might easily lead to erroneous conclusions, but when a large number of organisms of similar properties give constant results some conclusion may be warranted.

The work attempted here was to show whether any pathogenicity was demonstrable in the hemolytic streptococci obtained from milk. Davis (2) reports some pathogenicity for rabbits in a few cultures, but his results are generally negative.

Most of the cultures isolated were inoculated into mice. This was done as soon as possible after isolation. As soon as their purity was assured the organisms were given three daily transfers in serum broth, then 0.5 c.c. of the third broth culture was inoculated into the peritoneal cavity of a mouse. The human pathogens were treated in the same way. All mice were observed daily for seven days and doubtful results were repeated. In no case were any of the typical milk strains pathogenic for mice. One of the "W" strains killed mice and all of them seemed to cause rather severe symptoms. The back would be arched, the hair roughed, and respirations rapid and labored. This seems to confirm the suspicion that these strains are of the pyogenic type and resemble more closely the human pathogenic than the milk strains. All of the former group showed definite pathogenicity for mice (see Tables X and XI). In every case the mice which died

TABLE X.

Results of mouse inoculations with pathogenic cultures.

Culture.	Time after inoculation.				
	18 hours.	24 hours.	36 hours.	7 days.	Septicemia.
24	Normal	Normal	Normal	Normal	
177	Normal	Normal	Normal	Normal	
179	Normal	Normal	Normal	Normal	
180	Normal	Normal	Normal	Normal	
182	Dead				+
200	Dead				+
233	Dead				+
234	Dead				+
238	Sick	Dead			+
240	Dead				+
242	Sick	Dead			+
260	Dead				+
250	Dead				+
252	Dead				+
258	Sick	Dead			+
259	Dead				+
261	Dead				+
247	Sick	Sick	Dead		+
136 (*vir.*)	Normal	Normal	Normal	Normal	
33	Dead				+
7	Sick	Sick	Dead		+
18	Normal	Normal	Normal	Normal	
44	Dead				+
DB	Dead				+
LI (*vir.*)	Sick	Dead			+
Repeated tests					
24	Sick	Dead			+
177	Sick	Dead			+
179	Sick	Sick	Dead		+
180	Sick	Normal	Normal	Normal	
252	Dead				+
234	Dead				+
240	Sick	Dead			+
242	Sick	Sick	Dead		+
186 (*vir.*)	Sick	Normal	Normal	Normal	

TABLE XI.

Results of mouse inoculations with milk cultures.

Culture.	Time after inoculation.				
	18 hours.	24 hours.	36 hours.	7 days.	Septicemia.
U 1	Normal	Normal	Normal	Normal	
U 2	Normal	Normal	Normal	Normal	
U 3	Normal	Normal	Normal	Normal	
U 4	Normal	Normal	Normal	Normal	
F 7	Normal	Normal	Normal	Normal	
F 8	Normal	Normal	Normal	Normal	
F 9	Normal	Normal	Normal	Normal	
F10	Normal	Normal	Normal	Normal	
W 1	Sick	Sick	Sick	Normal	
W 2	Sick	Sick	Sick	Normal	
W 3	Sick	Sick	Sick	Normal	
W 4	Sick	Sick	Normal	Normal	
W 5	Sick	Sick	Sick	Normal	
W 6	Dead				+
W 8	Normal	Normal	Normal	Normal	
W 9	Sick	Sick	Sick	Normal	
C 7	Normal	Normal	Normal	Normal	
C 8	Normal	Normal	Normal	Normal	
C 9	Normal	Normal	Normal	Normal	
C10	Normal	Normal	Normal	Normal	

were autopsied and the heart blood and peritoneal fluid streaked on blood agar plates and inoculated into broth. The organisms thus isolated were always identical morphologically and in their hemolytic properties with those injected. It would seem then that the milk strains were not pathogenic for mice. A number of them were injected intravenously into rabbits with entirely negative results.

Heineman (12) reported that S. lacticus could be made virulent for rabbits by successive subcutaneous inoculations. The organism was re-isolated from the site of inoculation and re-injected into another rabbit and so on. The lesions were said to become more severe until a culture was obtained, after several transfers, which killed rabbits. Although the culture was examined and identified after each isolation, the danger of involving a streptococcus from the body of the animal is not excluded.

Typical milk strains of hemolytic streptococci were injected successively into six mice. The mouse was etherized, in each case, two days after the inoculation and the streptococci isolated from the peritoneal fluid, and occasionally from the heart blood. These were reinjected into the next mouse and so on. One-half c.c. of broth culture was injected into each mouse. The organisms showed no signs of increasing in virulence, the mice in all cases appearing normal. It does not seem possible, therefore, to render a strain of the hemolytic streptococci from milk virulent by passage through mice.

THERMAL DEATH RATES.

The statement is made by Ayers, Johnson, and Davis (13) that pathogenic streptococci are killed at 60° C., when held for 30 minutes in milk and that if any survive this heating, they are so attenuated as not to be dangerous. Some of their cultures which survived this temperature, although coming from pathological sources, were not considered pathogenic. The question is thus raised as to whether or not there is a decided difference in the thermal death rate of the pathogenic and non-pathogenic streptococci. If true, this would settle any doubts in regard to efficiency of pasteurization with respect to streptococci, and furthermore, might offer a convenient means of differentiation between the pathogenic and non-pathogenic streptococci in milk.

Some recent publications have emphasized the fallacy of speaking of thermal death points when all of the factors governing the complete sterilization of the culture are not controlled. It has been repeatedly demonstrated that the time required to kill all the organisms at a given temperature is dependent on the initial number present. According to Phelps (14), "the rate of dying whether under the influence of heat, cold, or chemical poison, is unfailingly found to follow the logarithmic curve of the velocity law, if the temperature be constant." This curve never reaches a zero value, although approaching it indefinitely. He further states that thermal death points "are merely accidental, depending in the first place wholly on the technique adopted and again upon the chance of the last few survivors living or dying, when for a considerable length of time these chances are about equal."

Any method, therefore, which is designed to give a refined comparison between the effect of heat upon different cultures must take into consideration the initial number of organisms and compare, not the temperature at which all organisms are killed in a given time, but the rate of death at a given constant temperature. In other words, the velocity coefficients of the rates of death of the organisms should be compared. Bruett (15) used these velocity coefficients, or K values, determined for spore bearing organisms, before and after "cold shock,'' in order to determine the utility of ''blanching'' in food canning. This method made possible a refined comparison of the rates of death of cultures of the same species when differently treated. Differences in heat resistance between the various groups of streptococci ought, therefore, to be demonstrable by this method. It is conceded that the determination of the thermal death point will show differences in heat resistance among streptococci, but in this determination only complete sterilization is considered. The accidental survival of a single or a few organisms is not distinguished from the survival of all or a large proportion of the initial number and thus distinct differences in behavior of two organisms at a given temperature may be obscured.

It has been sufficiently demonstrated that bacteria when subjected to fixed unfavorable conditions, such as a constant high temperature, will die off in a manner corresponding to the law of monomolecular reactions. This law is expressed by the equation:

$$K = \frac{1}{t} \log \frac{B}{b}$$

in which K is a constant, the velocity coefficient of the rate of death,

t is the time between determination of number of organisms.

B is the initial number of bacteria in any time interval.

b is the number of bacteria at the end of time t.

In this formula K varies only with the temperature, is independent of the initial number of organisms, and does not involve complete sterilization. The point of complete sterilization is indefinite, therefore the K is more accurately determined when b is not near the point of complete sterilization. The best results are obtained, according to Phelps (53), when there is about a 50 per cent. reduction in the number of organisms.

It was thought best to determine the velocity coefficients of the rate of death of the hemolytic streptococci in milk, since the application of the results to pasteurization as well as to the differentiation of the organisms, was sought. Each strain was grown in milk for three days, transfer being made each day. The third 24-hour culture was used for the test. The heating was done in a De Khotinsky electric bath in which the temperature was controlled very accurately. A tube containing ten c.c. of sterile milk was placed in the bath and held for five minutes. This was found sufficient to raise the milk to the temperature of the bath. The organisms were then pipetted into the tube from the 24-hour culture. It was found that with the milk cultures one tenth of a cubic centimeter of the 24-hour culture, added to the heated milk tube, gave a suspension containing between one and ten million bacteria per c.c. With the pathogenic strains one c.c. of the 24-hour culture gave about the same number. The latter, therefore, did not seem to develop so abundantly in milk. The difference in counts, however, may have been due in part to the presence of longer chains in the pathogenic strains, with the result that more individuals formed the seed of a single colony.

After the organisms were added the tube was shaken and one c.c. removed for the initial count. The milk strains were enumerated in dextrose infusion agar, but it was found necessary to use whole blood agar to count the pathogenic strains. The colonies were so small on the infusion medium that there was danger of overlooking some of them, while on blood agar the zones of hemolysis around each colony made counting easy. Four plates were made for each count. Two dilutions were plated, each one in duplicate. Counts which did not check satisfactorily, both in the two dilutions and in the duplicates of each, were discarded and the determination repeated.

The tubes while being heated were kept immersed to a point at least two inches above the level of the milk. Before each count the milk was mixed by rolling the tube vigorously between the palms, as far as possible without removing the tube from the water bath. Care was taken to avoid splashing the milk high up on the walls of the tube or on the cotton plug, as in this way some of the organisms might escape being heated to the required temperature.

The test of the applicability of the law of monomolecular reactions to this work is the constancy of the K values at a given temperature. A number of preliminary determinations were made at seven and one half minute intervals with a group of cultures, in order to test the constancy of the K values. The results are best shown by graphs. If the logarithms of the numbers of organisms determined at various intervals, in a culture held at constant temperature, are plotted against time, a line may be drawn, the slope of which is dependent upon K. If the K values are constant for determinations at all intervals of time, the organisms are dying at a constant rate, and the plot should therefore be a straight line. By plotting the logarithms of the numbers, we may test the constancy of K without determining it. Graphs plotted from some of the data are shown in Fig. 1.

It will be seen that the *K* values do not vary greatly, as most of the plots approximate a straight line. Subsequent determinations with other cultures did not always yield such uniform results, but the variations from straight lines are quite similar in all and can be explained on the same basis.

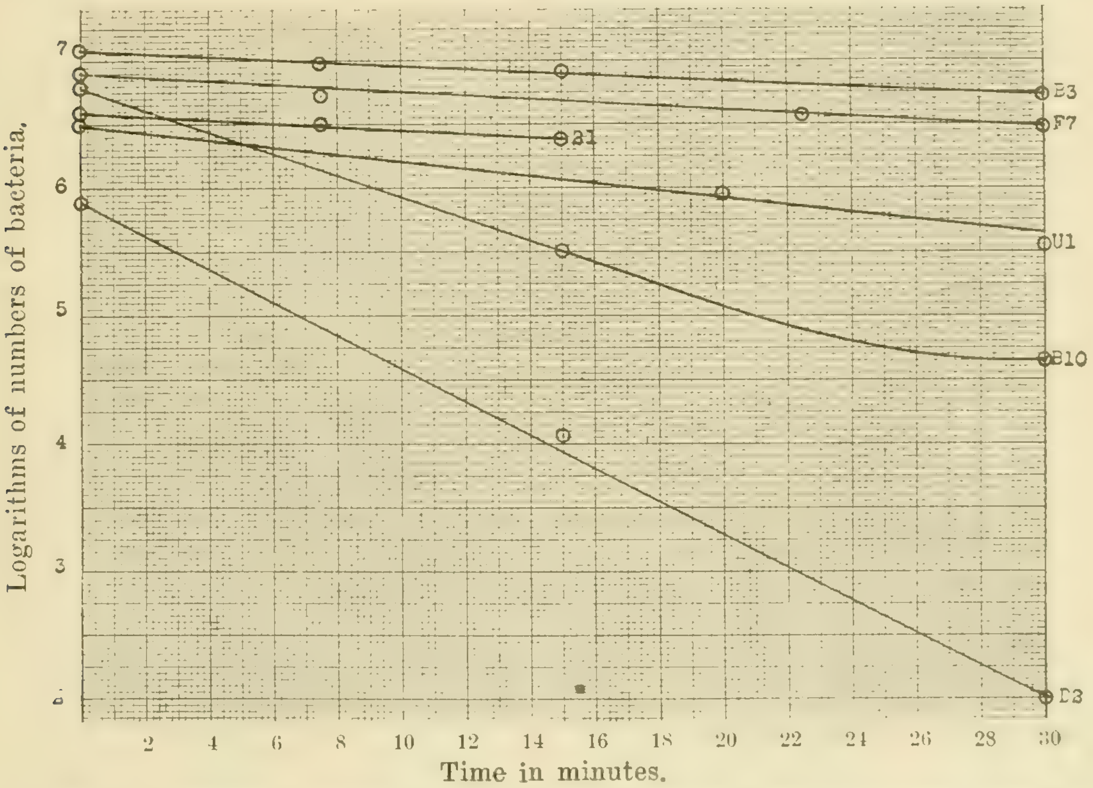

FIG. 1. Graphs showing thermal death rates of hemolytic streptococci in milk at 60° C.

It frequently occurs in these that the velocity coefficient of the rate of death, during the first interval of time, is not as great as in the second and third, also that as the number of organisms gets very small there is considerable variation in the coefficient. The most constant results are obtained when the decrease in the number of organisms does not go much beyond fifty per cent. This may be due to the greater percentage of experimental error in counting small numbers of organisms, or perhaps the law is no longer strictly operative when small numbers are reached.

The slow rate in the first interval of time has two possible explanations. First, a slight error is introduced by the addition of the suspension of the organisms to the heated milk, causing a slight reduction in temperature. The first counts were made as soon after the introduction of the organisms, as the heated milk suspension could be properly mixed. In this way, the sample may have been taken occasionally before the proper temperature was regained. Second, shaking after the first heating interval may cause the breaking up of chains, making a larger number of colonies for the actual number of bacteria present than appeared in the first count.

Although the K's are not constant for all intervals of time, it was considered that the best comparison of the rates of death of two groups of cultures could be obtained by comparing the K's computed for the longest practicable length of time. Most of the K's for the milk strains were, therefore, computed for 30 minutes and those of the pathogenic strains for a ten minute period.

It becomes apparent from an examination of the data in Tables XII–XIII that there is a very marked difference between the behavior of the human and milk hemolytic streptococci at 60° C. The human strains die very rapidly and reach a very small number per c.c. in ten minutes, while the milk strains die very slowly at this temperature; some, in fact, appear not to decrease to any appreciable extent in thirty minutes. It is questionable, because of the small number of organisms left after the time interval, whether or not

TABLE XII.

Rates of death of milk cultures in milk at 60° C.

Culture.	Initial number of bacteria per c.c.	Number of bacteria per c.c. after			
		10 minutes	20 minutes	30 minutes.	K.
U 1	9,700,000			1,000,000	.0329
U 2	3,300,000	2,100,000	930,000	344,000	.0327
F 7	8,450,000	7,550,000	6,600,000	5,170,000	.0071
U 5	9,900,000	9,800,000	9,900,000	9,800,000	.0614
U 4	11,260,000	11,800,000		10,100,000	.0014
F 8	5,200,000	4,300,000	3,800,000	3,300,000	.0056
F 9	4,400,000	4,400,000	3,900,000	3,400,000	.0036
C 7	4,300,000	4,200,000	4,000,000	5,900,000	.0014
C 8	9,000,000	140,000	90,000	20,000	.0884
C 9	7,100,000	2,900,000	1,600,000	1,000,000	.0270
C10	7,000,000	2,900,000	1,390,000		.0651
F11	5,600,000	1,600,000	180,000	25,000	.0783
F12	4,500,000	4,200,000	3,400,000	2,700,000	.0073
W 4	3,900,000	27			.5139
W 6	2,500,000	240			.4017
W 8	36,300,000	86			.5628
W 2	4,100,000	0			
W 3	530,000	110			.3662
W 9	3,400,000	107			.4201

the *K* values for the pathogenic streptococci represent the rapidity of the rate of decrease. The curve of decline of numbers probably reaches a point, before the ten minutes have elapsed, where death is no longer in strict accordance with the law of monomolecular reactions, or in other words, reaches that small number in which chance plays an important part in survival. The determination of the *K*'s, however, obviously gives a better picture of the behavior of the organisms than a determination of the point of complete sterilization—the indeterminable point at which all of these few remaining organisms have died.

TABLE XIII.

Rates of death of pathogenic cultures in milk at 60° C.

Culture.	Initial number of bacteria per c.c.	Bacteria per c.c. after ten minutes.	K.
177..............	3,000,000	100	.5221
250..............	5,200,000	60	.4957
258..............	4,300,000	191	.3400
182..............	610,000	47	.3257
230..............	1,460,000	11,400	.2038
259..............	5,000,000	540	.3986
100..............	480,000	0	
261..............	3,900,000	310	.4099
242..............	11,500,000	266	.4637
254..............	360,000	3,500	.2011
253..............	6,600,000	56	.5192
240..............	300,000	345	.2939
179..............	765,000	53	.4159
260..............	4,700,000	101	.4667
247..............	900,000	16	.4750
252..............	255,000	2,500	.2008
24..............	930,000	14	.4822
200..............	3,200,000	24	.5124

The rates of death of the pathogenic strains are fairly uniform as compared with the milk strains although none of the typical strains of either have a death rate which approaches that of the other group. The "W" strains, as in previous tests, show great similarity to the pathogenic strains. In all of the tests they have been identical with the pathogenic group and there seems to be little reason to believe that they are not of this group. None of these organisms were obtained from pasteurized milk but from milk produced under the best sanitary precautions. It would be difficult to prove whether or not these strains could be pathogenic to man, but their similarity to pathogenic strains and the fact that they were found only in unpasteurized milk, argues very strongly for pasteurization of all milk regardless of the care used in producing it.

The great difference in the thermal death rate of pathogenic human and non-pathogenic milk streptococci suggests that this character might be added to the list of characters which serve to differentiate them. It is one which can easily be determined with simple apparatus. It is also a character which perhaps reflects the habitual environment of the organism, that is, a purely saprophytic organism would be more likely to withstand wide ranges of temperature than one which lives and grows largely at the temperature of the human or other animal body.

The question as to whether pathogenic streptococci might survive pasteurization arose in connection with these experiments. The usual pasteurizing temperature is 145° F. or 62.8° C., although 60° C. is occasionally used. Ayers, Johnson, and Davis (13) concluded that pathogenic streptococci will not survive this temperature for 30 minutes.

All of the pathogenic strains used in the above tests were held at 60° C. in milk for 30 minutes. All strains were tested several times. The tubes were cooled after heating and incubated for 48 hours at 37° C. Occasionally some of them survived, grew in the milk, and were carefully identified. In these tests the initial number of organisms was the same as that in the determination of the death rate, that is, 1 c.c. of a 24-hour milk culture was added to a tube containing about 10 c.c. of milk at 60° C. Some of the cultures which grew after the one heat test failed to grow after a second test. Others, namely, 33, 44, DB, and LI (S. viridans), grew quite consistently after heating for 30 minutes at 60° C. If the initial number of organisms was reduced to 0.1 c.c. of a 24-hour milk culture, none of the cultures survived the test.

The question arose whether the organisms lost their virulence by the heating. To test this, hemolytic culture No. 33 and viridans culture LI were inoculated into mice. Both mice died in 24 hours. The organisms were isolated from the heart blood on blood agar plates and identified. A single colony of each was transferred to a milk tube and incubated for 24 hours. One c.c. was transferred from each of the tubes to sterile milk tubes at 60° C. and held for 30 minutes. The tubes were then cooled and incubated for 24 hours. 0.5 c.c. of each was injected intraperitoneally into a mouse and a third mouse was inoculated with 0.5 c.c. of sterile milk. Both of the mice, inoculated with the heated streptococci cultures, died within 48 hours, while the control survived. The organisms were again isolated from the heart blood and identified. This simple experiment seems to show that the virulence of the streptococci which survive pasteurization is not lost, or at least they are able to produce virulent cells in the same milk in which they were heated. The experiment was repeated with the same results.

It must be remembered that in these experiments the number of organisms present was very large. There were probably from one to five million bacteria per c.c. present at the beginning. One would scarcely expect to find milk so highly contaminated with pathogenic streptococci. However, the margin of safety in pasteurization would be much more adequate if higher pasteurizing temperature were used.

It was thought that repeated heating of a culture might lead to the selection of a heat resistant strain of the organisms. Accordingly two hemolytic cultures, No. 33 and DB, and viridans culture LI were pasteurized at 60° C. for 30 minutes, every second day for a time. It was intended to heat them ten times and then to determine their death rates and compare them with those of the original cultures to see if the heat resistance of the organisms had increased. None of the three cultures, however, survived ten heatings; the viridans strain was killed by the sixth heating, the hemolytic strain No. 33, by the eighth, and DB by the ninth. It seems clear that the cultures did not gain in heat resistance by the repeated heatings. The apparent decrease in resistance after a number of heatings could not be explained.

Conclusions.

The number of cultures used in this work was not great, especially the group of human pathogenic cultures, yet they were carefully selected and are believed to be fairly representative. Several conclusions may, therefore, be warranted from the study of the data presented:

1. Hemolytic streptococci which differ in many respects from human pathogenic streptococci occur quite generally in raw and pasteurized milk. A large number of these strains behaved alike in a series of tests, and they therefore seem to form a homogeneous group or species.

2. There are certain general differences in morphology between milk and human hemolytic streptococci. These are not absolute and by themselves are of little value for purposes of differentiation.

3. The reaction of milk hemolytic streptococci in litmus milk is

characteristic and is of importance in differentiating them from pathogenic strains.

4. The pathogenic hemolytic streptococci have a greater range of fermenting power than do the milk strains. The most constant difference between the two groups is the failure of the latter to ferment saccharose.

5. The identification of hemolytic streptococci should always be confirmed by testing for hemolysis in broth culture. The true Beta type produces hemolysin in broth, while others which resemble the Beta type quite closely do not.

6. There is no constant difference in the hemolytic titer of milk and pathogenic strains, but the time required for hemolysis to take place is distinctly greater with the milk strains. This characteristic could be used to aid in differentiation.

7. The milk hemolytic streptococci are not pathogenic for mice, and virulence cannot be demonstrated even by successive passages.

8. The difference in the thermal death rates of milk and pathogenic hemolytic streptococci serves to differentiate them. The former die at a slow rate in milk at 60° C., while the latter die rapidly.

9. With a very large number present it is possible for pathogenic streptococci to survive pasteurization at 60° C. for 30 minutes. The number of survivors is undoubtedly very small, but they are able to produce a culture virulent for mice. Higher pasteurizing temperatures would give a better margin of safety.

10. Successive heating of pathogenic streptococci does not result in the production of a heat resistant strain.

11. Hemolytic streptococci resembling the pathogenic type in every respect were isolated from certified milk. They are of low heat resistance and could readily be destroyed by proper pasteurization.

BIBLIOGRAPHY.

1. Smith, T. and Brown, J. H.
1915. A study of streptococci from certain presumably milk-borne epidemics of tonsillitis occurring in Massachusetts, J. Med. Research, XXXI, 445.

2. Davis, D. J.
1918. Further observations on hemolytic streptococci in milk, J. Med. Research, XXIII, 559.

3. Brown, J. H.
1920. The cultural differentiation of Beta hemolytic streptococci of human and bovine origin, J. Exper. Med., XXXI, 35.

4. Esten, W. M.
1909. Bacterium lactis acidi and its sources, Conn. Storrs Agr. Exp. Sta., Bul. 59.
5. Sherman, J.
1918. Some characters which differentiate lactic-acid streptococcus from streptococci of the pyogenes type occurring in milk, J. Bact., III, 153.
6. Evans, Alice C.
1914. A study of the streptococci concerned in cheese ripening, J. Agr. Research, XIII, 235.
7. Andrews, T. W. and Horder, T. J.
1906. A study of the streptococci pathogenic for man, Lancet, II, 708, 775, 852.
8. Holman, W. L.
1916. The classification of streptococci, J. Med. Research, XXXIV, 177.
9. Savage, W. G.
1906–1908. The milk of apparently healthy cows with particular reference to the presence of leucocytes and of streptococci, Rept. of Med. Off. to Loc. Govt. Bd., 1906–1907, 206 and 1907–1908, 359.
10. Houston, A. C.
1904–1905. The bacteriological examination of fresh cow dung, Rept. of Med. Off. to Loc. Govt. Bd., 326.
11. Broadhurst, Jean.
1913. The effect of meat and of meat extract media upon the fermentative activity of streptococci, J. Infect. Dis., XIII, 404.
12. Heineman, P. G.
1915. The variability of two strains of streptococcus lacticus, J. Infect. Dis., XVI, 221.
13. Ayers, S. H., Johnson, W. T. and Davis, D. J.
1918. The thermal death point and limiting H-ion concentration of pathogenic streptococci, J. Infect. Dis., XXIII, 291.
14. Phelps, E. D.
1911. The application of certain laws of physical chemistry in the standardization of disinfectants, J. Infect. Dis., VIII, 27.
15. Bruett, Eva M.
1919. Utility of blanching in food canning. Effect of cold shock upon bacterial death rates, J. of Indus. and Eng. Chem., II, 37.

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A STUDY OF THE FACTORS WHICH INTERFERE WITH THE USE OF YEAST AS A TEST ORGANISM FOR THE ANTINEURITIC SUBSTANCE.

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In a recent paper Williams[1] described a method of testing for the presence of the antineuritic substance which in this laboratory has been generally referred to as water-soluble B. For this purpose yeast was employed, and the basis of the test was the assumption, verified by experimental trials by Williams, that the yeast cell is capable of multiplication only when the antineuritic substance is present in its nutrient medium. By means of a series of dilutions of solutions which he used for the cultivation of yeast he was able to obtain results which gave promise of his method being useful in a quantitative sense as well as qualitative. Owing to the fact that the test as described by Williams could be carried out with very small amounts of material, and in a period of time much shorter than is required for the demonstration of the presence of the antineuritic substance in a nutrition experiment on a mammal, the method proposed for utilizing yeast as an experimental organism seemed to offer great possibilities of usefulness to those who are engaged in the study of methods for the isolation and identification of this most interesting dietary factor.

The work reported in this paper had its origin in an effort to develop proficiency in the conduct of experiments with yeast by the Williams method.

Considerable inconvenience was experienced in carrying out the test, owing to the number of small particles which became detached from the pen, and which interfered with the counting of the number of cells. A modification of Williams' method was therefore introduced, in which a platinum syringe needle, having an opening at right angles to its axis, was used for depositing the droplets on the cover-slip. The needle was attached to a piece of glass tubing by means of a short piece of rubber tubing, and it was found that with this instrument the suspension of yeast cells could be easily and quickly distributed, without leaving any detritus in the droplets. It was found that a fairly uniform distribution of cells could be obtained by this method. The slides were incubated in the manner described by Williams.

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[1] Williams, R. J., *J. Biol. Chem.*, 1919, xxxviii, 465.

Our results were characterized by lack of uniformity in the development of the yeast, and for this reason an attempt was made to substitute for the cover-slips used by Williams the ordinary blood counting chamber of the Levy type. In order to break up the clumps of yeast cells without injuring their vitality air was blown through the suspension instead of shaking the latter. The solution was then distributed in test-tubes, 5 or 10 cc. in each. The results by this technique were much more uniform. Table II shows on one hand how the cells frequently were distributed in the droplet method, and on the other how the same suspension behaved in the blood counting chamber method.

Williams called attention in his paper to the fact that many times it is impossible to count the number of cells after a period of growth on account of the fact that the cells grow in three dimensions. He found it necessary to count a part and estimate the probable number. By the counting chamber method it is not necessary to guess at the number of cells. If the suspension is too rich it may be diluted to such a point as facilitates the counting. Immediately before counting, a little formalin was added to the solution in order to kill the cells and avoid further growth. Using this technique we have attempted to test several substances known to be rich or poor in the dietary factor water-soluble B. We will first consider the results obtained by the extraction of wheat germ with hot water.

1 gm. of wheat germ was boiled with 50 cc. of water for 10 minutes (Extract 1), the residue was boiled again with 50 cc. of water for 10 minutes (Extract 2), and the second residue was boiled with 50 cc. of water for 10 minutes (Extract 3). The separation of the residue from the soluble part offered some difficulty due to the viscosity of the solution. In some cases we

have separated the solution from the undissolved part by means of a centrifuge and in others by filtration in an alundum crucible. Only by passing the solution through a Berkefeld filter could an approximately clear solution be obtained. Eight successive extractions were made.

These extracts were evaporated to the volume of 25 cc. so each 1 cc. corresponded to 0.04 gm. of wheat germ. 5 cc. of the control solution were put into test-tubes previously sterilized, and the first extract was added as shown in Table I.

TABLE I.

Tube No.	Control solution.	First extract.	Control solution.
	cc.	*cc.*	
1	5.0	0	Seeded with Fleischmann's yeast.
2	5.0	0.1	
3	5.0	0.2	
4	5.0	0.3	
5	5.0	1.0	

From these tubes we made a series of slides by the droplet method and ran a comparative series by the counting chamber method, the results of which are presented in Table II.

If we take into account only those droplets which contain one single cell, we observe that they give rise to very different numbers of cells by the end of an 18 hour incubation period. In those squares where the yeast cell was dead, or had reached the stage where budding had ceased, there was, of course, no multiplication.

Seeding 1.

1 cell gave rise to 3 cells.
1 " " " " 14 "
1 " " " " 27 "
1 " " " " 34 "
1 " " " " 62 "

Seeding 2 (Control Solution Plus 0.1 cc. of Wheat Germ Extract).

1 cell gave rise to 3 cells.
In the rest the single cells were dead or incapable of budding.

TABLE II.

*Yeast Cells Counted in Different Droplets.**

Tube 1.					Tube 2.					Tube 3.					Tube 4.					Tube 5.				
Before incubation.																								
1	2	**1**	0	0	0	**1**	2	2	**1**	**1**	2	**1**	**1**(?)	0	2	**1**	**1**	0	0	1	D†	**1**	0	0
D	3	0(?)	0	0	3	2	D(?)	3	2	2	**1**	3	2	0	3	**1**	2	2	D	0	2		3	0
1	2	2	D(?)	**1**	**1**	**1**	**1**	0(?)	2	**1**	2	2	0	0	2	**1**	**1**	2	**1**	0	**1**	2	**1**	3
D	1	**1**	**1**	**1**(?)	**1**	2	2	0	**1**	2	3	0	**1**	2	3	0	**1**	0(?)	**1**	0	0	**1**	2	**1**
0	**1**	**1**	**1**(?)	**1**	0	3	2	**1**(?)		**1**	**1**	2	**1**	2	4	0	**1**	0	0	**1**	2(?)	**1**	2	0
After incubation for 18 hrs.																								
1	500	**0**	0	0	0	**1**	0	130	0	**207**	252	**301**	**0**	0	2	**1**	**245**	0	0	**500**	500	**1**	0	
27	3	2	0	0	0	2	143	6	98	258	**1**	0	0	0	0	**1**	2	500	0	0	5			
1	2	0	0	0	**1**	**1**	0	254	143	**101**	2,000	0	0	0	2	**1**	**0**	1,000	**1,000**	0	**0**	342	**1,000**	500
19	**1**	**3**	**62**	**0**	**3**	2	31	0	**0**	2	290	0	**151**	1,500	440	0	**130**	500	**349**	0	0	**1,000**	0	**290**
0	**3**	**27**	**34**	**14**	0	0	41	**0**		**0**	**124**	394	**0**	300	1.000	0	**400**			**500**		**75**		0

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* In those cases where we were successful in seeding with a single cell the entry is made in bold-faced type. In the table showing the growth of the cells, the bold-faced type represents the progeny of single cells.

† D = Dead.

Seeding 3.

1 cell gave rise to 101 to 301 cells.
2 cells " " " 252 " 2,000 "

Seeding 4.

1 cell gave rise to 130 to 1,000 cells.

Seeding 5.

1 cell gave rise to 75 to 1,000 cells.

On the other hand, when using the test-tubes which contain 5 to 10 cc. of control solution, to which different amounts of wheat germ extract were added as stated above, by using the counting chamber we have a greater chance of securing the same number of cells distributed evenly, as respects their vital conditions, and thus effect a diminution of the magnitude of error due to individual cell differences. This procedure, in fact, gave more comparable results.

TABLE III.

Tube No.	First count.	Second count (after 18 hrs.).	Average.
	cells	*cells*	*cells*
1	21	23	22
2	132	92	112
3	214	198	206
4	342	308	325
5	602	684	643

By the counting chamber method, and using the same suspension already used with the droplet method, the results given in Table III were secured.

The largest variation in the series of countings on the same suspension was in Tube 5 (602 to 684—difference 82, about 13 per cent variation between the first and second counting).

It seems, therefore, that a more even and reliable result can be obtained by the use of this procedure than with the droplet method devised by Williams, even though the droplets were made by the platinum needle instead of a pen.

We have tested the effect of increasing the quantity of the first aqueous extraction of wheat germ on the growth of yeast. The uniformly beneficial effects were suggestive that the high content of water-soluble B in the extract exerted a stimulating effect on the growth of the yeast.

The results of testing the second and third extracts prepared from the residue of the first extraction when tested for their effect on the growth of yeast are given in Tables IV and V.

TABLE IV.

Test with Second Extract.

	No. of cells.		Average after 12 hrs. incubation.
Control solution	21		21
" " + 0.1 cc.	132	92	112
" " + 0.2 "	214	198	206
" " + 0.4 "	342	308	325
" " + 1.0 "	602	684	643

TABLE V.

Test with Third Extract.

	No. of cells.		Average after 24 hrs. incubation.
Control solution	150	189	174 5
" " + 1.0 cc.	378	366	372
" " + 2.0 "	421	480	450 5
" " + 3.0 "	511	551	532 5
" " + 1.0 " first extract	1,068	1,240	1,154
" " + 1.0 " second "	649	633	641

The results show clearly that there is a stimulating effect of the extracts on the rate of multiplication of yeast and that in general the greater the amount of extract added the more pronounced the acceleration.

Alcoholic extracts of wheat germ were also prepared in which various strengths of alcohol were used, varying from 40 to 75 per cent. In all cases the germ was boiled for 10 minutes in alcohol and the solution filtered through paper or an alundum filter, then evaporated on a water bath at low temperature with a fan blowing on the surface. In all cases it was observed that the growth of yeast was markedly stimulated by the addition of an alcoholic extract of wheat germ to the control solution.

In some experiments we have used wheat germ extract treated with sodium bicarbonate and autoclaved, in order to destroy as much as possible of the antineuritic substance. Before addition of such extracts to the nutrient medium the sodium bicarbonate was neutralized with hydrochloric acid.

Extract of fresh beef made by boiling the latter with water was also employed. The beef muscle was treated with 2 per cent of $NaHCO_3$, moistened and allowed to stand for 3 hours, then autoclaved 1 hour at 15 pounds pressure. Extract of rolled oats was also used. The oats were treated with 2 per cent of $NaHCO_3$ and autoclaved at 15 pounds pressure as described for the meat muscle. The oats so treated were fed to young rats in a diet consisting of rolled oats 40.0, gelatin 5.0, casein 5.0, NaCl 1.0, butter fat 3.0, $CaCO_3$ 1.5 per cent. The animals failed to grow. After 41 days 3 per cent of untreated wheat germ was added to the diet to furnish the antineuritic substance. The animals responded at once with rapid growth. 40 per cent of untreated oats furnishes an abundance of antineuritic substance, water-soluble B, for growth. The treatment of the oats with alkali had, therefore, destroyed its content of this substance.

The series has been extended by the employment of meat extract prepared by precipitation with barium, the addition of barium being stopped while the solution was still distinctly acid. After the removal of the barium sulfate the remaining acidity was neutralized by ammonium hydroxide.

All extracts employed in our experiments were carefully sterilized by boiling and kept in an ice box until used. Observations were made to establish that there was no contamination of these extracts with bacteria.

Table VI illustrates the effect of 40 per cent alcoholic extract of wheat germ added to the control solution on the rate of development of yeast cells. Equal amounts of successive extracts were employed.

Table VII illustrates the effect of adding 1 cc. of each of a series of eight successive extracts of wheat germ, using 40 per cent alcohol as a solvent.

Table VIII illustrates clearly the progressively increasing benefit to the yeast by adding increasing amounts of 75 per cent alcoholic extract of wheat germ.

TABLE VI.

	No. of cells after 18 hrs.		Average.
5 cc. of control solution	147	133	140
5 “ “ “ “ + 1 cc. first extract.	811	798	804.5
5 “ “ “ “ + 1 “ second “	507	509	508
5 “ “ “ “ + 1 “ third “	392	416	404
5 “ “ “ “ + 1 “ fourth “	430	399	414.5

TABLE VII.

	No. of cells after 20 hrs.			Average.
5 cc. control solution	570	433	480	494.3
5 “ “ “ + 1 cc. first extract	1,560	1,720		1,640
5 “ “ “ + 1 “ second “	1,610	1,720		1,665
5 “ “ “ + 1 “ third “	890	810		850
5 “ “ “ + 1 “ fourth “	760	670		715
5 “ “ “ + 1 “ fifth “	590	690		640
5 “ “ “ + 1 “ sixth “	820	680	780	760
5 “ “ “ + 1 “ seventh “	760	560		660
5 “ “ “ + 1 “ eighth “	500	600		550

TABLE VIII.

	No. of cells after 20 hrs. incubation per 400 squares.		Average.
5 cc. control solution + 1.0 cc. water	499	483	491
5 “ “ “ + 0.9 “ “ + 0.1 cc. first extract	1,000	870	935
5 cc. control solution + 0.8 cc. water + 0.2 cc. first extract	1,360	1,390	1,375
5 cc. control solution + 0.6 cc. water + 0.4 cc. first extract	1,610	1,740	1,675
5 cc. control solution + 1.0 cc. water + 1.0 cc. first extract	2,180	2,540	2,360

Tables IX, X, and XI illustrate the effect of adding to the control solution aqueous or alcoholic (40 per cent alcohol) extracts of wheat germ. In these experiments the wheat germ had

TABLE IX.

	No. of cells after 18 hrs. per 400 squares.		Average.
5 cc. control solution	770	580	675
5 “ “ “ + 1 cc. autoclaved wheat germ extract	2,460	1,860	2,160
5 cc. control solution + 2 cc. autoclaved wheat germ extract	2,650	3,530	3,090
5 cc. control solution + 1 cc. alcoholic extract of wheat germ	2,520	2,420	2,470
5 cc. control solution + 2 cc. alcoholic extract of wheat germ	2,530	2,320	2,425

TABLE X.

	No. of cells after 18 hrs. per 400 squares.		Average.
5 cc. control solution	160	210	185
5 “ “ “ + 1.5 cc. water + 0.5 cc. 75 per cent alcoholic extract	1,340	1,210	1,275
5 cc. control solution + 1.0 cc. water + 1.0 cc. 75 per cent alcoholic extract	2,060	2,030	2,045
5 cc. control solution + 1.0 cc. water + 0.5 cc. 75 per cent alcoholic extract + 1.0 cc. autoclaved wheat germ	1,920	1,480	1,700
5 cc. control solution + 1 cc. autoclaved wheat germ + 1 cc. 75 per cent alcoholic extract	2,790	3,920	3,355
5 cc. control solution + 1 cc. autoclaved wheat germ + 1 cc. water	2,190	2,120	2,155

TABLE XI.

	No. of cells after 20 hrs. per 400 squares.		Average.
5 cc. control solution + 2 cc. water	100	120	110
5 “ “ “ + 1 “ “ + 1 cc. extract autoclaved wheat germ	2,440	2,320	2,380
5 cc. control solution + 2 cc. extract autoclaved wheat germ	2,730	2,630	2,680
5 cc. control solution + 1 cc. water + 1 cc. 40 per cent alcoholic extract wheat germ	2,040	2,320	2,180
5 cc. control solution + 2 cc. 40 per cent alcoholic extract wheat germ	2,190	1,670	1,930

been treated with 2 or 4 per cent sodium bicarbonate, allowed to stand in a moist condition for 3 hours or longer, in order that the alkali might penetrate the material. The wheat germ was then autoclaved at 15 pounds pressure for 3 hours, the acid neutralized, and the material dried in a current of warm air.[2]

TABLE XII.

	24 hrs.		48 hrs.	
	Counts.	Average.	Counts.	Average.
1. 10 cc. control solution.	300 310	305	2,350 2,500	2,425
2. 10 " " " + 1 cc. 10 per cent glucose.	390 330	360	5,020 4,940	4,980
3. 10 cc. control solution + 2 cc. 10 per cent glucose.	360 380	370	8,300 7,650	7,975
4. 10 cc. control solution + 1 cc. autoclaved wheat germ extract.	1,540 1,350	1,445	2,880 3,060	2,970
5. 10 cc. control solution + 1 cc. autoclaved wheat germ extract + 1 cc. 10 per cent glucose.	1,400 1,390	1,395	3,780 4,020	3,900
6. 10 cc. control solution + 1 cc. 75 per cent alcoholic wheat germ extract.	1,650 1,810	1,730	5,340 5,450	5,395
7. 10 cc. control solution + 1 cc. 75 per cent alcoholic wheat germ extract + 1 cc. 10 per cent glucose.	1,570 1,540	1,555	5,400 5,760	5,580

It is easily apparent that the addition of these extracts exerted a profound stimulating effect on the growth of the yeast, but that the extent of acceleration of the growth of yeast is not proportional to the amount of extract added.

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[2] This germ was used in a feeding experiment with rats to show whether it still contained any appreciable amount of water-soluble B. The ration consisted of casein 18, treated wheat germ 15, butter fat 5, salt mixture (No. 185) 3.7, dextrin 58.3 per cent. The animals failed to grow. In a subsequent period rapid growth took place when 3 per cent of untreated germ was included in the diet. All the antineuritic substance had been practically destroyed by heating with alkali.

Table XII illustrates the effect on the growth of yeast of adding to the control solution varying amounts of glucose and of glucose together with extracts of wheat germ.

It will be observed that Nos. 2 and 3 show a surprising acceleration due to the addition of double the amount of glucose. 1 cc. of 10 per cent glucose solution added to 10 cc. of control solution produced 4,980 yeast cells after 48 hours. 2 cc. of the glucose solution added to 10 cc. of control solution led to the development of 7,975 cells in the same interval. Comparing Nos. 4 and 5, the addition of 1 cc. of autoclaved wheat germ extract produced 2,970 cells in 48 hours, whereas the same medium with 1 cc. of 10 per cent solution produced 3,900 cells. That results are not always consistent is shown by comparison of Nos. 6 and 7, in which there was a marked effect from the addition of 1 cc. of 75 per cent alcoholic extract of wheat germ, and very slight further stimulation by the addition of 1 cc. of 10 per cent glucose.

Table XIII illustrates further the lack of uniformity in the development of yeast under different conditions.

Comparing Nos. 2 and 3, greater growth was secured by adding to 10 cc. of the control solution 1 cc. of 10 per cent glucose solution than was obtained by the addition of 0.1 cc. of 75 per cent alcoholic extract of wheat germ without additional glucose. In order to test the possibility of securing comparable development of yeast when the cultural conditions for every cell were as nearly as possible identical the following procedure was tried.

A drop of yeast suspension was placed on a clean cover-slip, and the number of yeast cells contained in it carefully determined by counting. The yeast cells were then immediately washed into a sterile test-tube by means of control solution, and the amount of control solution was then adjusted so that 0.1 cc. of nutrient solution was available for each yeast cell present. The following five experiments illustrate the results of this procedure. An examination of Table XIV shows that in the first test-tube there were 21 yeast cells, each with 0.1 cc. of nutrient medium. After 12 hours the number was increased to 39, an approximate multiplication of the original number by 2. In the second tube 29 cells were present, and in 12 hours this had multiplied the original number by 16. In the third tube 31 cells were present, and after 12 hours this had multiplied the original number by 19.

In the fourth tube 34 cells were present, and after 12 hours this had multiplied 21½ times the original number. In the fifth tube 43 cells were present and in 12 hours the original number was multiplied by 10. The cause of these irregular results is unquestionably to be attributed to the introduction of injured cells or cells of low reproductive power into the test specimens.

TABLE XIII.

	18 hrs.		52 hrs.	
	Counts.	Average.	Counts.	Average.
1. 10 cc. control solution.	350 290	320	4,680 4,100	4,390
2. 10 " " " + 1.0 cc. 10 per cent glucose.	420 380	400	4,660 4,620	4,640
3. 10 cc. control solution + 0.1 cc. 75 per cent alcoholic extract wheat germ.	620 670	645	3,600 3,760	3,680
4. 10 cc. control solution + 0.1 cc. 75 per cent alcoholic extract wheat germ + 1.0 cc. 10 per cent glucose.	910 980	945	6,260 5,320	5,790
5. 10 cc. control solution + 0.5 cc. 75 per cent alcoholic extract wheat germ.	940 1,300	1,120	4,240 4,400	4,320
6. 10 cc. control solution + 0.5 cc. 75 per cent alcoholic extract wheat germ + 1 cc. 10 per cent glucose.	930 890	910	4,340 4,452	4,396
7. 10 cc. control solution + 1 cc. 75 per cent alcoholic extract wheat germ.	1,250 1,800	1,525	4,420 4,590	4,505
8. 10 cc. control solution + 1 cc. 75 per cent alcoholic extract wheat germ + 1 cc. 10 per cent glucose.	1,130 1,240	1,185	4,380 4,190	4,285

We have sought to test the value of a procedure involving the addition of 1 drop of opalescent suspension of yeast cells in water to several test-tubes, each containing 10 cc. of nutrient solution, and then counting these cells in the Levy chamber before incubation.

Table XV illustrates one set of experiments in which fairly close agreement was obtained by this procedure, but the agreement is presumably due to the good fortune of selecting a series of cells all or nearly all of which were capable of development.

In Table XVI are recorded the results of a series of trials designed to determine the effect of various extracts on the rate of development of yeast. The volume of solution was kept constant by the addition of glucose solution or water. The materials from which the extracts were prepared in these experiments had been subjected to heat under pressure in the presence of sodium bicarbonate as described in the methods of preparation of materials. In all cases these preparations had been tested with rats and found to be at least practically free from the dietary factor water-soluble B. Notwithstanding the fact that they con-

TABLE XIV.

Tube No.	Original count.	Control solution.	Count after 12 hrs.	Average.
	cells	*cc.*	*cells*	*cells*
1	21	2.1	43 35	39
2	29	2.9	464 461	462.5
3	31	3.1	594 586	590
4	34	3.4	767 698	732.5
5	43	4.3	422 454	438

TABLE XV.

Tube No.	Count per 400 squares.	Count after 22 hrs.		Average.
	cells	*cells*		*cells*
1	3	186	198	192
2	4	174	195	184.5
3	3.5	169	153	161

tained so little of this substance as to be undemonstrable by experiments on the rat the extracts were still very effective in stimulating the growth of yeast. These results suggest that although the addition of the antineuritic substance in minute amounts may have influenced the growth of yeast in these experi-

TABLE XVI.

		No. of cells after 18 hrs.		Average.
1.	9 cc. control solution. 1 " 10 per cent glucose solution. 1 " H_2O.	390	350	370
2.	8 " control solution. 2 " glucose " 1 " H_2O.	310	380	345
3.	8 " control solution. 2 " glucose " 1 " eighth extract wheat germ.	320	340	330
	8 " control solution. 2 " dextrose " 1 " autoclaved wheat extract (first).	1,750	1,610	1,680
5.	8 " control solution. 2 " glucose " 0.5 " first extract wheat germ. 0.5 " H_2O.	1,880	1,750	1,815
6.	8 " control solution. 2 " glucose " 1 " first extract wheat germ.	1,960	1,990	1,975
7.	8 " control solution. 2 " glucose " 1 " rolled oats extract (made from rolled oats autoclaved with 2 per cent $NaHCO_3$).	1,980	2,010	1,995
8.	8 cc. control solution. 2 " glucose " 1 " beef extract (from beef steak autoclaved with 2 per cent $NaHCO_3$).	1,700	1,760	1,730

TABLE XVII.

		No. of cells after 20 hrs.		No. of cells after 43 hrs.		Gas.
		Counts.	Average.	Counts.	Average.	
						cc.
1.	9 cc. control solution.	156	146.5	2,510	2,695	2.5
	1 " 10 per cent glucose solution.	137		2,880		
	1 " H_2O.					
2.	8 " control solution.	143	141	2,300	2,240	2.5
	2 " glucose "	139		2,180		
	1 " H_2O.					
3.	8 " control solution.	133	130.5	1,740	1,875	0.5
	2 " glucose "	128		2,010		
	1 " eighth extract wheat germ.					
4.	8 " control solution.	732	732	4,180	4,205	10
	2 " glucose "			4,230		
	1 " autoclaved wheat extract (first).					
5.	8 " control solution.	493	514	3,900	4,340	4
	2 " glucose "	535		4,780		
	0.1 " first extract wheat germ.					
6.	8 " control solution.	675	675	5,020	5,115	5
	2 " glucose "			5,210		
	0.5 " first extract wheat germ (alcohol).					
	0.5 " H_2O.					
7.	8 " control solution.	1,340	1,340	4,740	4,420	8
	2 " glucose "			4,100		
	1 " first extract wheat germ (alcohol).					
8.	8 " control solution.	170	156.5	4,830	4,985	8
	2 " glucose "	143		5,140		
	1 " fifth extract wheat germ (alcohol).					
9.	8 " control solution.	636	694	3,680	3,995	8
	2 " glucose "	752		4,310		
	1 " extract rolled oats (fresh oats).					
10.	8 " control solution.	1,027	1,027	4,560	4,750	10
	2 " glucose "			4,940		
	1 " steak extract (alkalinized steak).					
11.	8 " control solution.	880	880	2,230	2,285	3
	2 " glucose "			2,340		
	1 " third extract wheat germ (alcohol).					

ments, this factor is so small as to make it practically certain that other added substances, as glucose and amino-acids, also served to influence the results in a marked way.

Table XVII illustrates the results obtained in a series of experiments in which it was sought to estimate by the gas produced by the yeast culture the extent of growth of the yeast in several

TABLE XVIII.

	No. of cells after 20 hrs.		Average.
1. 1 cc. control solution.	1,020	1,230	1,125
2. 1 " " " 1 " autoclaved wheat extract.	1,980	2,080	2,030
3. 10 " control solution. 0.5 " digested meat (prepared from fresh steak not autoclaved with alkali).	1,820	1,970	1,895
4. 10 cc. control solution. 0.5 " digested meat extract (prepared from fresh steak not autoclaved with alkali).	2,048	2,200	2,124
5. 10 cc. control solution. 1 " digested meat extract (prepared from fresh steak not autoclaved with alkali).	2,412	1,940	2,176
6. 10 cc. control solution. 1 " first extract wheat germ.	2,020	2,380	2,200
7. 10 " control solution. 0.5 " first extract wheat germ.	1,680	1,880	1,780

types of modified nutrient medium. These results are so variable, when we compare the gas production with the number of yeast cells generated during the period of incubation, that the measurement of the amount of gas produced does not give promise of value in this line of work.

In Table XVIII is presented a series of observations on the effect of adding an amino-acid mixture obtained by the hydrolysis of beef muscle with sulfuric acid.

So far as our knowledge permits us to judge, these digested meat extracts could serve only as a source of amino-acids, since the treatment was sufficient to reduce the proteins practically to the amino-acid state, and, since muscle tissue is, even in the fresh condition, very poor in the antineuritic factor (water-soluble B), the same meat after hydrolysis with strong sulfuric acid would scarcely contain a trace of it. Yet these meat digests exerted a profound influence on the rate of development of yeast, approximately doubling the number of cells which were counted after 20 hours incubation in Experiments 6 and 7, Table XVIII. It is again apparent that doubling the amount of the antineuritic substance derived from wheat germ extract does not exert a proportional stimulating effect on the growth of yeast.

The series of observations which we have presented above seem to us to form a conclusive demonstration that the use of yeast as a test organism for determining the presence or absence of the antineuritic dietary factor is complicated by so many disturbing factors as to make it of little if any value. Whenever extracts of natural foods are to be tested it is inevitable that food substances of one kind or another which greatly stimulate the growth of yeast must be added simultaneously with the unidentified dietary essential for which the test was designed. Glucose and amino-acids effect such stimulation

We do not desire to draw the deduction from the data here presented that the antineuritic substance is not necessary for the development of yeast, or that it does not stimulate the growth of yeast, but the several modifications of the procedure originally described for the utilization of yeasts as a test for the antineuritic substance point so definitely to several difficulties in the way of making this a satisfactory test that it has seemed to us desirable to present the results for the benefit of those who may be interested in this line of work.

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STUDIES ON EXPERIMENTAL RICKETS.

I. THE PRODUCTION OF RACHITIS AND SIMILAR DISEASES IN THE RAT BY DEFICIENT DIETS.

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PLATES 2 AND 3.

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Students of the etiology of rickets have advanced the most diverse views to account for its occurrence. Heredity, dietetic factors, faulty hygienic conditions, infections of a microbial or other nature, and disturbances of the endocrine glands have all been incriminated as the causative agents.

In the conduct of nutritional experiments on the rat which have been carried on by the members of the Department of Chemical Hygiene of the Johns Hopkins University for more than a decade, it has been the custom to autopsy all animals which have died from any cause. In the course of these routine autopsies pathological conditions of the skeleton, *viz.* enlargement of the epiphyseodiaphyseal junction, fractures of the ribs, enlargement of the costochondral junctions and vertebral ends of the ribs, abnormal spinal curvatures, etc., have been observed repeatedly and have been regarded as evidences of rickets. Other problems prevented for a time any effort at intensive study of the nature of the factor or factors which operate to produce these conditions. There was never any room for doubt that the diet was the sole determining factor in the etiology of the lesions in question, for the entire colony of approximately 2,000 animals lived under identical conditions except for those related to the composition and source of their food.

Although the members of the Department of Chemical Hygiene were of the opinion that the skeletal malformations found in their rats were the result of rickets, it was not possible for them alone to arrive at definite conclusions owing to a lack of the necessary pathological experience. It so happened that the Department of Pediatrics had been engaged for a number of years in the study of rickets from the chemical and pathological standpoints and had already conducted experiments with a view to the production of rickets in animals. It seemed advisable, therefore, for the two departments to unite in a cooperative study concerning the origin and nature of the lesions which had been produced in the rat as the result of the varied and presumably insufficient diets. In this undertaking the Department of Chemical Hygiene is responsible for the planning of the diets which have produced the disease, and their administration, the care of the animals, and the interpretation of the nature of the faults in the food mixtures themselves. The Department of Pediatrics has assumed the responsibility for the determination of the exact nature of the pathological processes and has undertaken the interpretation of them in the light of human osteopathology and in so far as it is possible the correlation of the osteo- and organopathology to the dietary defects. Each department has utilized the information gained from the other so as to plan most effectually for future experiments.

The present paper, the first of a series, has for its purpose the presentation of certain faulty diets which when fed to the young rat produce several disturbances in the growth of the skeleton which have in common fractures, enlargement and distortion of the costochondral junctions, vertebral deformity, the overproduction of osteoid tissue, and irregularities in the calcification of the intercellular substance of the proliferative cartilage or absence of lime salt deposits from the matrix of this tissue. Administration of some of these diets to young rats results in the development of pathological changes in the osseous system which are identical with those found in the bones of rachitic children. Others of the dietary formulas used caused changes in the bony and cartilaginous skeleton which bear a close resemblance to those found in human rickets but can only be said to be similar and not identical with this condition. One of these diets causes in the second generation of rats maintained upon it an extreme degree of osteoporosis with multiple fractures but with relatively slight irregularity in the calcification of the cartilage.

The study which we have undertaken is a most comprehensive one. It has already involved the feeding of several basal rations of a faulty nature supplemented by the addition of single and multiple purified food constituents. This plan originated in the Laboratory of Chemical Hygiene and has been employed for some years in that laboratory as an effective biological method for the analysis of the nature of the deficiencies of the various natural foodstuffs. More than 300 modifications of diet have been effected in producing the rachitic and the related pathological conditions in the skeleton of the rat, and in demonstrating the nature of the faults in the food mixtures. The work of making histological examinations of the rats representative of all the experimental groups has been most time-consuming and is as yet far from being completed.

The present paper is a preliminary communication. We reserve for later papers, therefore, descriptions of the diets and growth curves and the exact pathological conditions produced in the skeleton by each of the diets in question.

In order to appreciate the view we have come to hold relative to the etiology of rickets, the following diets are described and the nature of the defects is pointed out.

No. 2249.

	per cent
Whole wheat kernel	30.0
" maize "	20.0
Polished rice	12.0
Rolled oats	11.5
Peas	12.0
Navy beans	12.0
$NaHCO_3$	1.5
NaCl	1.0

This diet is faulty in that it contains decidedly less than the optimum of both the dietary factors, fat-soluble A and calcium. It is by no means free from either of these essentials. The quality of the protein while not of the best cannot be a factor of importance in inducing malnutrition on this diet.

No. 2581.

	per cent
Beef liver (steamed and dried)	20.0
Casein	10.0
NaCl	1.0
KCl	1.0
Dextrin	65.0
Butter fat	3.0

This diet contains an abundance of protein of good quality and all the inorganic elements necessary for the normal nutrition of the rat except calcium, which is present in very small amount. It is rich in fat-soluble A and contains a fairly liberal amount of water-soluble B. This diet contains a considerable excess of potential acidity. Low calcium and acidity (after catabolism) are the only known faults in this food mixture.

Nos. 2661 and 2677.

	per cent
Rolled oats	40.0
Flaxseed oil meal	8.3
NaCl	1.0
$CaCO_3$	1.5
Dextrin	49.2

This diet is satisfactory for fairly good growth in the rat when supplemented with 5 per cent of butter fat. It has but one serious fault therefore; *viz.*, lack of fat-soluble A. Its proteins are not of a very high biological value but the two seeds when supplemented as they are in this diet with NaCl and $CaCO_3$ are capable of supporting good growth.

No. 2638.

	per cent
Whole wheat kernel	30.0
" maize "	30.0
Polished rice	10.0
Rolled oats	10.0
Peas	10.0
Navy beans	10.0

This diet of cereals and legume seeds is poor in Ca, Na, and Cl ions and also poor in fat-soluble A. Its ash is somewhat acid.

No. 2733.

	per cent
Whole wheat kernel	30.0
" maize "	19.5
Polished rice	9.5
Rolled oats	8.5
Peas	8.5
Navy beans	8.5
Round steak (beef)	10.0
NaCl	1.0
$NaHCO_3$	1.5
Cod liver oil	3.0

Ration 2733, which is essentially the same as No. 2810 but with 1.5 per cent of sodium bicarbonate in place of the dextrin and 3.0 per cent of cod liver oil in place of the butter fat, enables the animals to develop in what appears from their growth curves and external appearance to be a fairly normal manner. The animals so produced have very poor skeletons, however, and the females frequently suffer complete breakdown and death when they attempt to nurse the young.

No. 2810.

	percent
Whole wheat kernel	30.0
" maize "	14.5
Polished rice	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Round steak (beef)	10.0
NaCl	1.0
Dextrin	1.5
Butter fat	5.0

This diet of cereal grains, legume seeds, steak, sodium chloride, and butter fat is satisfactorily constituted for the nutrition of the rat except for its low calcium content. When supplemented with 1.5 per cent of calcium carbonate, growth is normal and health is maintained over a long period of adult life. Successful nutrition of five successive generations of a family of rats has been secured with this food. Without the calcium addition, notwithstanding a high content of butter fat, normal calcification in the bones does not take place.

No. 2811.

	per cent
Bolted flour	30.0
Corn-meal	20.0
Rice	12.0
Rolled oats	11.5
Peas	12.0
Navy beans	12.0
$NaHCO_3$	1.5
Dextrin	1.0

Ration 2811 contains three degerminated products, wheat flour, corn-meal, and polished rice. These are degerminated and decorticated products of the endosperm. The oats, peas, and beans represent the entire seeds. The diet is too low in calcium, fat-soluble A, and protein, and failure of nutrition on this diet is due to these factors which are enumerated in the order of their importance.

No. 2815.

	per cent
Rolled oats	40.0
Gelatin	10.0
Salts (No. 185)	3.7
Dextrin	46.3

Ration 2815 is satisfactory except in that it is very poor in fat-soluble A. The protein is of good quality and the salt mixture is of such a nature as to supplement the deficiencies in the kernel.

No. 2869.

	per cent
Whole wheat kernel	28.0
" maize "	20.0
Polished rice	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Round steak	10.0
NaCl	1.0
$CaCO_3$	3.0

This diet is too low in fat-soluble A and too high in calcium. When fed with 2 per cent of cod liver oil the above diet induces normal development of young rats.

No. 2883.

	per cent
Liver	20.0
Casein	10.0
KCl	1.0
NaCl	1.0
Dextrin	66.0
Cod liver oil	2.0

Ration 2883 is satisfactorily constituted except for an extremely low calcium content. Liver is rich in fat-soluble A but in addition the diet contains 2 per cent of cod liver oil which is extremely rich in this factor.

No. 2911.

	per cent
Wheat	30.0
Maize	20.0
Rice	11.0
Rolled oats	11.5
Peas	11.0
Navy beans	11.0
NaCl	1.0
$NaHCO_3$	1.5
Butter fat	3.0

Ration 2911 consists of cereals and legume seeds supplemented with sodium chloride, sodium bicarbonate, and butter fat. In all cases the entire seed was used except rice, which was polished. The limiting factor in this diet for the nutrition of the rat during growth is the calcium content, which is too low. This food mixture with 1.5 per cent of calcium carbonate added is a fairly satisfactory diet for the nutrition of the rat throughout life. The protein is not of the highest quality but is sufficiently good to make possible normal development. The ash of any seed is acid and when seeds are metabolized there results an excess of acid ions in the body. Sodium bicarbonate is added to neutralize this acidity.[1]

Each of the diets described produces in young rats disturbances in the growth and formation of the skeleton. Each of these diets, when fed supplemented with purified food additions to make good the deficiencies noted in the descriptions of each, is capable of inducing approximately normal nutrition and normal skeletal growth. With these factors corrected there is in no instance any evidence from the general appearance of the animals of the deformities so characteristic of those restricted to the formulas as given in this paper.

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[1] In all cases our rations were fed finely ground and so mixed that the individual ingredients could not be picked out and eaten separately by the experimental animal.

The present discussion, as we have said above, is of necessity a preliminary communication and we are not now willing to hazard any statements in regard to the factors operating to produce rickets in the child or the experimental animal. It might seem from a perusal of the diets here discussed (representative of more than 300 formulas) that the cause of these diseases might lie in a deficiency of fat-soluble A or calcium in the food, or a disturbance in the metabolism of these factors. Many years experience with feeding experiments, however, have demonstrated to us how dangerous it is to draw conclusions from apparently obvious experimental data. Any suggestions regarding the absence of a specific antirachitic substance or deficiency of either fat-soluble A and calcium as the primary agent in the production of rickets would be ill considered and might be far from the truth. At present it is only possible to say that the etiological factor is to be found in an improper dietetic regimen. The large variety of dietary formulas the administration of which results in rickets and kindred affection gives abundant evidence of the complex nature of the causes operating in the production of the disease, and we shall attempt the presentation of their analysis in later papers.

EXPLANATION OF PLATES.

h, costochondral groove; *cc*, costochondral junctions; *cv*, costovertebral junctions; *f*, fractures; *d*, shaft; *met*, metaphysis; *os*, calcified bone; *o*, osteoid tissue; *c*, epiphyseal cartilage; *m*, bone marrow; *dia*, diaphysis; *ep*, epiphysis.

PLATE 2.

FIG. 1. Rachitic rat (R) contrasted with a normal animal (N) of the same age and sex (210 and 204 days respectively). The rachitic animal weighed 100 gm., the normal animal 295 gm.

FIG. 2. Pleural surface of the thorax from a case of severe experimental rickets showing the extreme enlargement at the costochondral junctions.

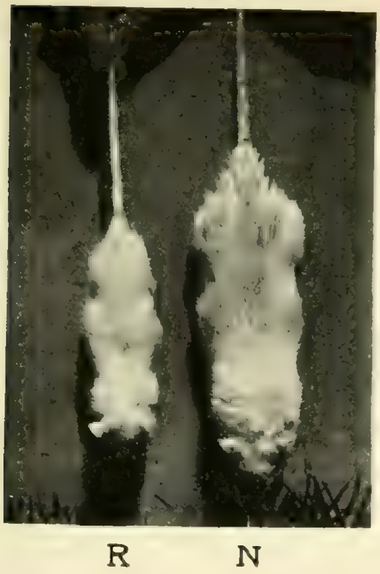
FIG. 3. An extreme case of experimental rickets. Cross-section of thorax at the level of the fifth costochondral junction. The cavity of the chest is almost completely divided into two parts by the bowed ribs and the enlargement of the costochondral junctions. Nearly the entire lungs were forced into the posterior chamber, the heart occupying the anterior chamber of the thorax. This section shows how much more marked the costochondral enlargement is on the pleural than on the external surface of the thoracic wall.

FIG. 4. Rachitic kyphoscoliosis. This picture shows also the enlargement of the ribs at the costovertebral junctions.

FIG. 5. Pleural surface of thorax from a case of experimental rickets to show multiple spontaneous fractures (healing) along the ribs.

PLATE 3.

FIG. 6. Photomicrograph of a section through the distal epiphysis and shaft of femur of a rat with experimental rachitis. The picture shows the overgrowth and irregular invasion of the epiphyseal cartilage, the presence of a metaphysis, and the overproduction of osteoid tissue. × 250.



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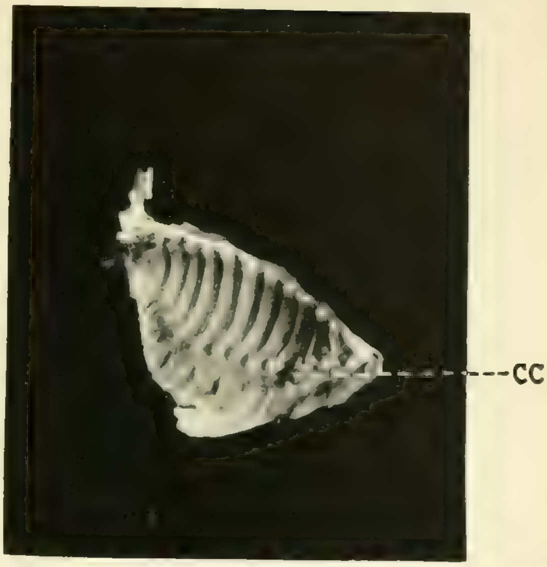

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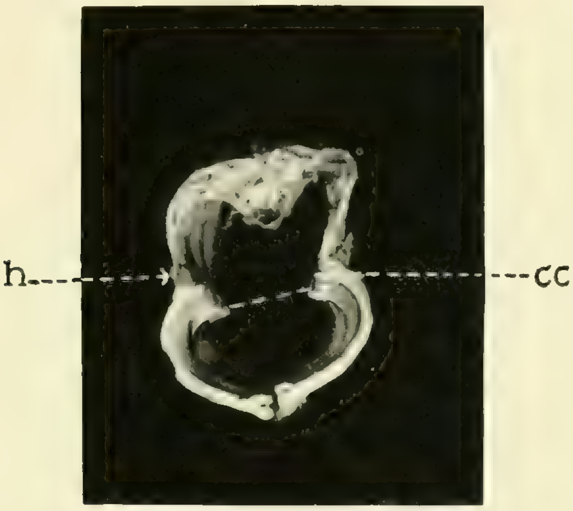

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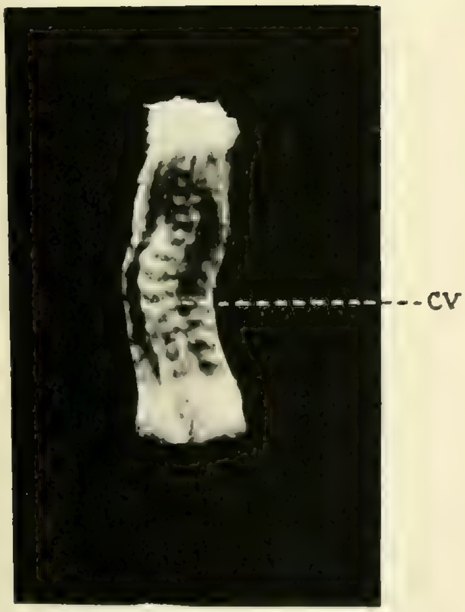

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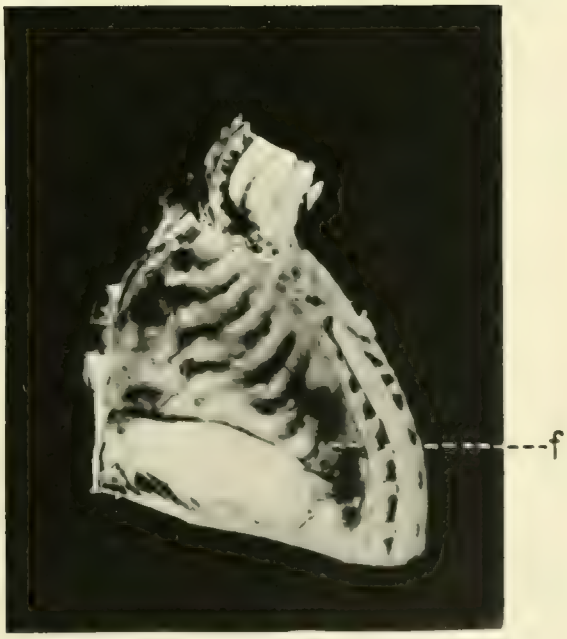

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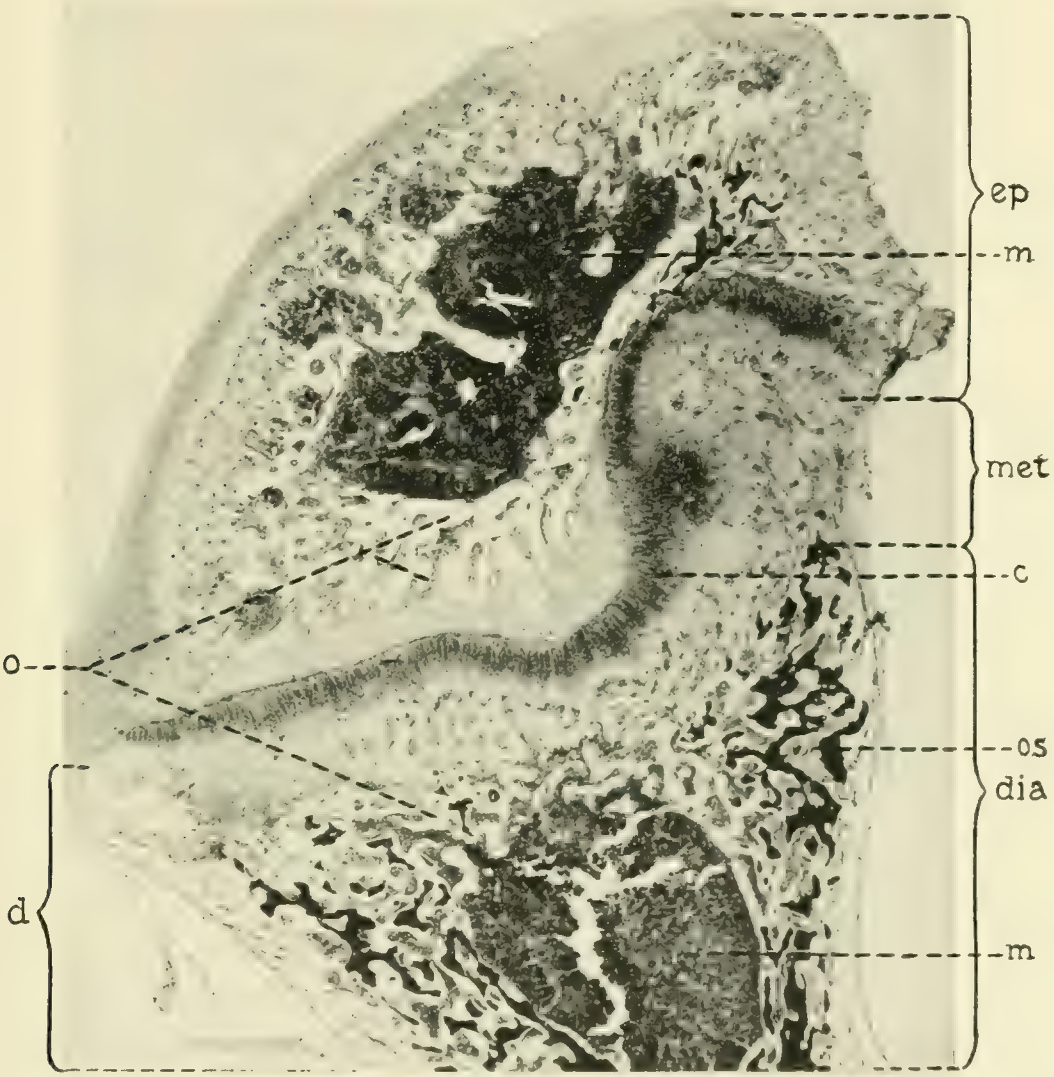
(McCollum, Simmonds, Parsons, Shipley, and Park: Experimental rickets. I.)



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(McCollum, Simmonds, Parsons, Shipley, and Park: Experimental rickets. I.)

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STUDIES ON EXPERIMENTAL RICKETS.

II. THE EFFECT OF COD LIVER OIL ADMINISTERED TO RATS WITH EXPERIMENTAL RICKETS.

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PLATES 4 AND 5.

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For many years it has been thought as the result of clinical experience that the administration of cod liver oil in rickets exerted a favorable influence on the course of the disease. During the past 15 years studies made on the metabolism of children suffering from rickets has made it reasonably certain that the administration of cod liver oil alters the calcium balance in such a manner that calcium will be retained in the body. The natural inference from both clinical observation and metabolic investigation was that cod liver oil increased the capacity of the skeleton to take up or hold calcium. The object of the experiments reported in this paper was to furnish direct proof that cod liver oil causes calcium salts to be deposited in the bones.

We were already familiar with the observation of Schmorl that, when healing occurs in the rachitic skeleton of the human being, the initial deposition of calcium at the cartilage-shaft junction of the long bones takes place not throughout the rachitic metaphysis or at random in it but on the epiphyseal side of the metaphysis in that zone of the proliferative cartilage in which calcium deposition normally occurs and presumably would have occurred had rickets never been present. Calcium deposits in the cartilage are easy to demonstrate by histological methods and the initial deposit in healing rickets, as seen in the sections taken longitudinally through the cartilage-shaft junctions, is situated in the lower part of the proliferative zone of cartilage. The lime salts are laid down along a line crossing the bone at right angles to its longitudinal axis and roughly paralleling the upper border of the proliferative zone, forming in the properly stained specimen a picture which cannot well be mistaken. Previous experience with the rat had taught us that by the use of faulty diets, especially certain diets deficient in the so called fat-soluble A or in both that substance and calcium, the cartilage and adjacent portions of the metaphysis of the long bones of the extremities could be rendered entirely free from calcium deposits and a condition identical with the rickets of human beings be obtained. A mode of approach to the problem at issue therefore appeared clear. If cod liver oil possessed calcium-depositing properties there seemed every reason to suppose that when added to the faulty diets of the rats whose epiphyseal cartilages and adjacent portions of the metaphyses had been rendered calcium-free, a lime salt deposit would be produced so characteristic as to have almost specific significance.

With the object of putting cod liver oil to the biological test fifteen young rats were placed on two deficient rations, Nos. 2677 and 2638. Eight animals were fed on Diet 2677 which, as will be seen from the formulas in Table I, is low in the fat-soluble A, and seven were placed on Diet 2638 which is a formula low in Ca, Na, and Cl ions, as well as in fat-soluble A. The administration of these diets it was hoped would render cartilage and the adjacent portion of the metaphysis entirely free from calcium salts.

The young rats listed as having received Diet 2638 may be said to have been on a deficient formula from their birth since the mother rats were maintained on the defective diet throughout the major part of the lactation period. Both lots of rats were maintained for from 50 to 80 days on the faulty rations.

Following the administration of the faulty diets the experimental animal ceased to gain, began to lose weight, and became extremely irritable, reacting to any stimulus with violent activity. About 40 days after the commencement of the administration of the faulty rations it was noted that those animals on Diet 2677 were beginning to develop xerophthalmia, which is characteristic for animals fed on diets deficient in fat-soluble A. When the condition of the animals had become such as to make it evident that life could be sustained only a few days longer, they were considered ready for the test administration of cod liver oil. To the food of four of the rats on Ration 2677, cod liver oil was added in such proportion that 2 per cent replaced 2 per cent of the dextrin in the ration. This modified formula was fed to the four animals for 2, 4, 7, and 8 days, respectively. To the food of the other four rats no cod liver oil was added and they were preserved as control animals. Of the seven rats on Ration 2638, three were selected as test animals. Cod liver oil was added to

TABLE I.

No. and sex of rat.	Diet and principal deficiency.		Age when killed.	Deficient diet administered.	Cod liver oil added to diet.	Weight after death.	Calcium deposit in cartilage.
		per cent	*days*	*days*	*days*	*gm.*	
248 ♀	Ration 2638:		75	70	5	49	+
249 ♀	Whole wheat kernel	30.0	75	75		66	0
250	" maize "	30.0	64	64			0
251	Polished rice	10.0	65	62	3	25	+
252	Rolled oats	10.0	67	67		31	0
253	Peas	10.0	67	62	5	43	+
254	Navy beans	10.0					
	Low in calcium, sodium, and chlorine and also poor in fat-soluble A. When cod liver oil was added to the diet 1 per cent replaced 1 per cent of maize.						
277 ♂	Ration 2677:		100	56		82	0
278 ♀	Rolled oats	40.0	110	41	2	64	+
298 ♀	Flaxseed oil meal	8.3	125	51	4	77	+
299 ♀	NaCl	1.0	110	68		61	0
306 ♂	$CaCO_3$	1.5	125	76		80	0
307 ♂	Dextrin	49.2	118	68	8	75	+
325 ♂	Low in the fat-soluble A. When		160	84		125	0
326 ♂	cod liver oil was added to the diet 2 per cent replaced 2 per cent of dextrin.		160	77	7	114	0

the food of these three so that 1 per cent replaced 1 per cent of maize and the three rats were fed on this formula for 3, 5, and 5 days, respectively. In the case of the four rats remaining which were being fed on Ration 2638 the original diet was continued unchanged. These rats also served as control animals for this diet. As the period of the test administration of cod liver oil came to an end the test animals and their respective controls were killed with chloroform and examined. The bones of test and control animals were compared for gross differences. The ribs, the lower ends of the femora, the upper ends of the tibiæ, the lower ends of the radii and ulnæ, and the upper ends of the humeri were then split longitudinally, placed in 10 per cent formaldehyde, and saved for histological examination. After fixation had taken place the lower end of the femur of each animal was sectioned with a freezing microtome without decalcification, stained with silver nitrate according to the method of von Kossa, and counterstained with hematoxylin and eosin. Blocks of tissue from the ends of all the bones mentioned were embedded in parlodion in some instances without decalcification, in others after short decalcification in Muller's fluid, and stained according to the silver nitrate, hematoxylin, and eosin methods just mentioned.

Microscopic study of the preparations so made permits the following generalizations: When the cartilage has become free from calcium as the result of the deficient diets, the addition of cod liver oil to the food for a period of from 2 to 7 days is followed by deposition of lime salts between the cells of the proliferative zone of cartilage. The deposit of calcium salts is linear, the width of the line apparently depending on the length of time during which the animal has been fed cod liver oil. The line of deposition is at right angles to the long axis of the shaft of the bone. The granular deposit seen in sections stained with hematoxylin does not invade the protoplasm of the cell body. Stained with silver nitrate the granules are colored dark brown or black. It is difficult to say whether or not there is a coincident deposition of calcium in the osteoid tissue of the shaft but from the appearance of the section it would seem that very little lime salt is laid down in the osteoid tissue until calcification of the proliferative cartilage is complete. No gross differences between the bones of the test and control animals were ever detected, and it was only upon microscopic examination that it was possible to note that in animals which had received no cod liver oil the epiphyseal cartilages were calcium-free. Those to whose diet the oil had been added showed a line of freshly laid down lime salt in the matrix of the proliferative zones. It will be seen from Table I that the cartilages and metaphyses of the bones of all the control animals on both diets used were successfully freed from lime salt deposits and that the administration of cod liver oil caused calcification of the matrix of the proliferative zones of cartilages of all rats fed on Diet 2638. Of the four rats which received cod liver oil after maintenance on Diet 2677 three showed calcification of the proliferative zone and one (No. 326) failed to react thus to the stimulus of the oil administration. It is difficult to assign a reason for the failure of this animal to react to the therapeutic agent but we are quite certain that the cause is to be sought with the animal rather than with the reagent itself. In a series of experiments such as those reported here there are always certain variable conditions encountered which are difficult of control. From among these might be cited variability in appetite or the presence of an absolute anorexia which is always a possibility to be reckoned when dealing with animals in poor physical condition. Again it is possible to bring an animal so near to death by prolonged feeding on a deficient diet that the organism may be without power to respond to remedial substances when they are finally received. These and other factors must be borne in mind in weighing the results of feeding experiments of this type, since the animals used must of necessity be in very poor general health before the restorative treatment is commenced.

The results of this series of preliminary experiments are twofold. They afford ocular and conclusive evidence of the specific beneficial effect of cod liver oil on rats suffering with experimental rachitis, in that some substance or substances in the oil cause calcium to be deposited in the same fashion in which deposition occurs in spontaneous healing of rachitis in man. Moreover, they prepare the way for the elaboration of a new test, which it is to be hoped may eventually prove even to be roughly quantitative, for the determination of the calcium-depositing potentiality of any substance in terms of cod liver oil units. It is especially interesting to note in the present experiments that calcium was deposited in the cartilages following the initiation of the cod liver oil treatment in spite of the fact that in some cases the calcium intake was far below normal. In view of the fact that in human cases of rickets the blood calcium is maintained throughout the disease at approximately the normal level it is interesting to speculate concerning the source from which the calcium deposited in the proliferative cartilage is derived.

This paper is in all senses a preliminary one and experiments are now under way with a view to perfecting the biological test here reported and to determining the calcium-depositing power of certain other possible therapeutic agents.

EXPLANATION OF PLATES.

ep, epiphysis; *d*, shaft; *met*, metaphysis; *dia*, diaphysis; *m*, bone marrow; *c*, epiphyseal cartilage; *ca*, calcified intracellular substance; *o*, osteoid tissue; *os*, calcified bone.

These photomicrographs were taken of sections cut from frozen tissue previously fixed in 10 per cent formaldehyde. The bone was not decalcified and the sections were stained by von Kossa's method and counterstained with hematoxylin and eosin. Calcified tissue is seen in the photographs as in the section colored black. × 250.

Plate 4.

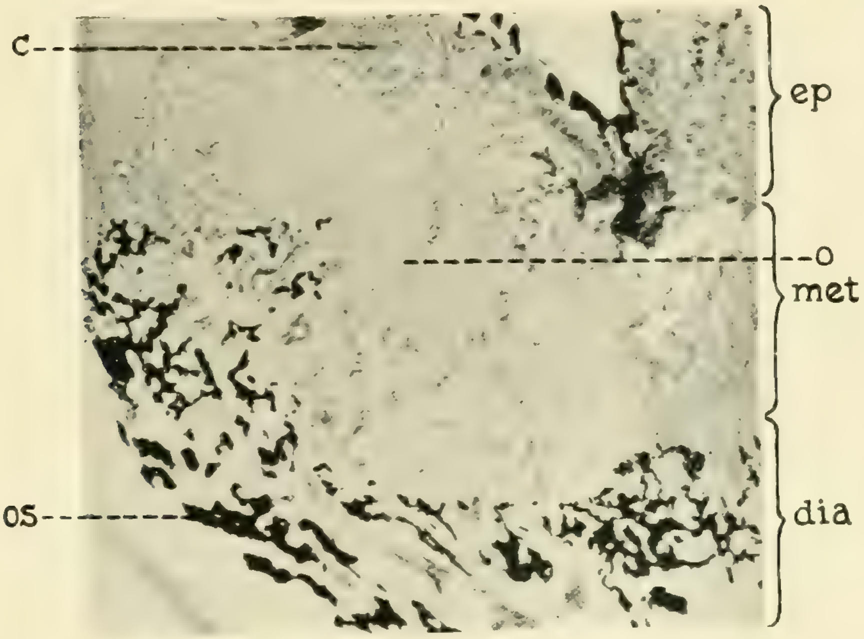
Fig. 1. Sagittal section through the lower ends of the femur of a rat fed for 84 days on Diet 2677. This picture shows besides complete absence of lime salts in the proliferative cartilage, the other cardinal microscopic signs of rickets; *viz.*, invasion of the cartilage and overproduction of osteoid tissue.

Fig. 2. Sagittal section from the distal end of the femur of a rat which was given cod liver oil after being maintained on Diet 2677. Beginning healing of experimental rickets is evidenced by the deposit of calcium (*ca*) in the proliferative zone of the epiphyseal cartilage, otherwise the bone is in the same condition as that in Fig. 1.

Plate 5.

Fig. 3. The animal from which the section shown here was made had lived on Diet 2638. The section shows marked evidences of rickets. The cartilage of the epiphysis is absolutely free from calcium salts.

Fig. 4. The rat from which this bone was taken had been given Diet 2638. *Five* days before death 1 per cent of cod liver oil was substituted for 1 per cent of maize in the ration. Note the heavy deposit of calcium (*ca*) in the epiphyseal cartilage resulting from cod liver oil administration.



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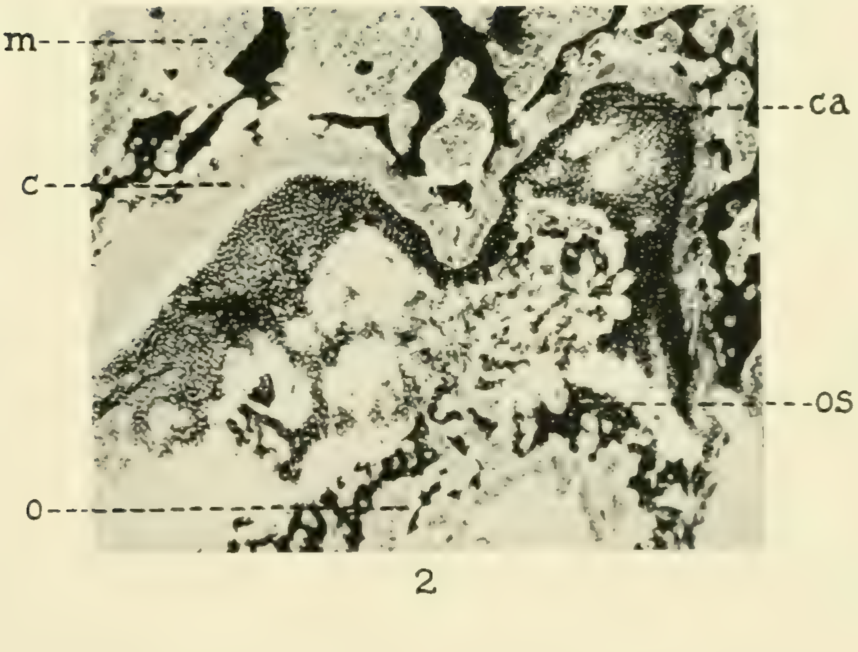

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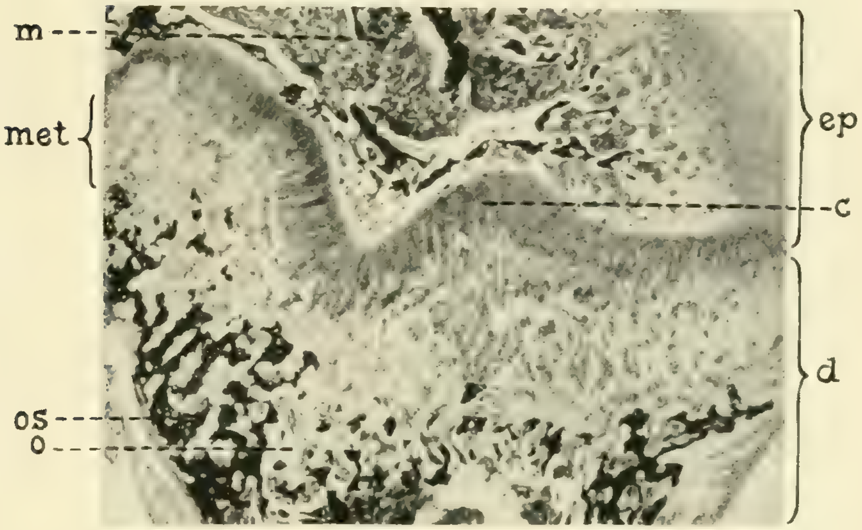
(Shipley, Park, McCollum, Simmonds, and Parsons: Experimental rickets. II.)



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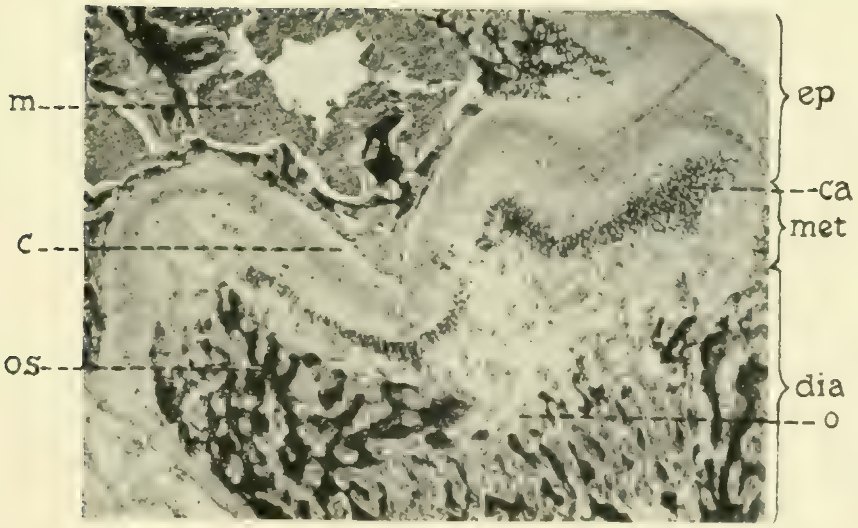

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[From The Johns Hopkins Hospital Bulletin, Vol. XXXII, No. 363,
May, 1921]

STUDIES ON EXPERIMENTAL RICKETS

III. A PATHOLOGICAL CONDITION BEARING FUNDAMENTAL RESEMBLANCES TO RICKETS OF THE HUMAN BEING RESULTING FROM DIETS LOW IN PHOSPHORUS AND FAT-SOLUBLE A: THE PHOSPHATE ION IN ITS PREVENTION

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As is now well known, Mellanby[1] has reported the production of rickets in puppies by diets deficient in their content of fat-soluble A,* or an antirachitic factor having a similar distribution. Unfortunately, it has not been possible to evaluate Mellanby's results by reading his published writings, since he has never supplied objective proof of the production of rickets in the form of illustrations showing the appearance of his specimens through the microscope. This omission has been unfortunate, inasmuch as the diagnosis of this disease can be made with certainty only on the evidence derived from microscopic changes in the bones. The gross deformities [160]

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* We are using provisionally the term fat-soluble A, to designate the organic factor which may play a special rôle in bone development. In a later paper we shall present further experimental data, of a nature which will serve better than those contained in the present discussion for deciding whether the organic factor contained in cod-liver oil, to which that substance owes its therapeutic value in certain diseases affecting the skeleton, is identical with the factor which we here refer to as fat-soluble A.

[160] which characterize rickets can be simulated in other pathological conditions, as, for example, the osteoporosis which develops in growing puppies during confinement in small cages. Evidence afforded by the X-ray is not completely convincing. X-ray examination fails to disclose the exact nature of the pathological process in operation, and to differentiate rickets, as it commonly occurs in children, from related conditions such as can be produced in animals through dietetic influence.

In a previous publication[2] we described 11 diets, some of which produced gross deformities resembling those seen in rickets. The nature of the faults of these diets was in all cases clearly pointed out. These diets included formulæ for food mixtures in which there was a deficiency of but one dietary factor, *i. e.,* calcium or fat-soluble A. The other diets described were faulty in the following respects: lack of fat-soluble A and excess of calcium, and low calcium and low fat-soluble A. Since the preparation of the earlier paper we have had occasion to observe also the effect of changing the plane of intake of the phosphate ion on the growing bone.

Experiments Designed to Show the Effects on the Skeleton of Diets Low in Their Content of Fat-Soluble A and Phosphorus

In order to bring out the effects of varying the plane of the phosphate ion in the diet on bone development, we have employed the following food mixtures:

Diet of Lot 2667

Rolled oats	40.0
Flaxseed meal	8.3
Sodium chloride	1.0
Calcium carbonate	1.5
Dextrin	49.2

100 gms. of this mixture contained 0.6714 gms. of calcium and 0.2165 gms. of phosphorus.

This diet is deficient to some extent in its protein content. The protein, both in amount and quality, is adequate, however, to induce growth, though at a rate distinctly below the maximum. But no development is possible on this food mixture unless it is supplemented by some substance which will serve as a source of the factor, fat-soluble A. This diet, as stated, is deficient in fat-soluble A, and also contains a relatively small amount of phosphorus. [160]

When young rats weighing from 20 to 70 gms. between 18 and 50 days old are confined to this diet, without the addition of any substance supplying fat-soluble A, they soon cease to increase in weight and then rapidly decline (Chart I). After an interval which varies markedly, depending principally on the age of the animal but to some extent on individual resistance, they all develop xerophthalmia. In our extensive experience no animal has ever recovered from this condition unless a substance capable of acting as a source of fat-soluble A was added to its diet. On the addition of such a substance, *e. g.*, butter fat or cod-liver oil, animals suffering from xerophthalmia steadily improve and return to normal health. While recovery is taking place, growth is resumed. With this diet we have conducted scores of experiments; the general behavior of the animals placed upon it and the development of xerophthalmia have occurred with unvarying regularity as described. [161]

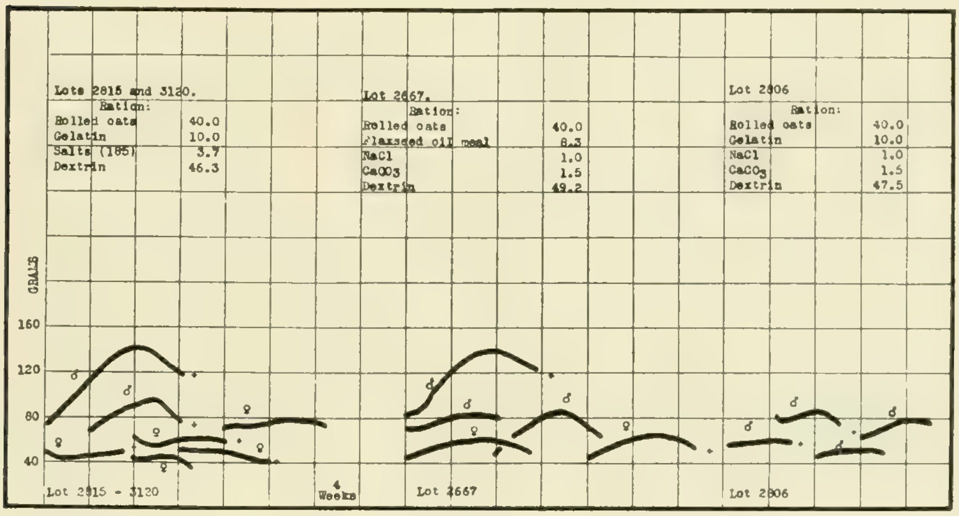

CHART I.

The Curves in this Chart Illustrate the Weight Changes in Young Rats Fed the Diets Described in the Text.

Diet of Lot 2806

Rolled oats	40.0
Gelatin	10.0
Sodium chloride	1.0
Calcium carbonate	1.5
Dextrin	47.5

100 gms. of this mixture contained 0.6414 gms. of calcium and 0.1580 gms. of phosphorus.

This diet contains protein of good quality and in sufficient amount to enable young rats to grow at a fairly rapid rate, provided the faults in the diet are corrected by suitable additions. The principal defect in this diet is a deficiency in fat-soluble A. Without the addition of this factor no growth is possible, and severe xerophthalmia develops followed by death. A second defect in the diet sufficiently grave to interfere in a measure with growth is the lack of sufficient phosphate ion. While growth can take place on the formula tabulated above, supplemented with some substance acting as a source of fat-soluble A, it is not so satisfactory as when the sodium chloride and calcium carbonate are replaced by a salt mixture containing not only these substances but suitable amounts of the phosphate ion as well.

Methods.—All the animals in the experiments were killed with ether. After careful postmortem examination various bones and some of the soft tissues were saved for histological examination.

In preparing bones for microscopical examination we have usually used the method recommended by Pommer—decalcification in Müller's fluid for periods ranging from two to fourteen days after preliminary fixation in formalin. The bones were dehydrated, embedded in celloidin and the blocks cut into sections from 10 to 60 microns thick. While we have used thionin and other dyes and have treated a large number of sections with silver nitrate after the method of Von Kossa, no staining technique has proved to be so satisfactory as that in which a combination of Ehrlich's hæmatoxylin with water soluble eosin is used. We have controlled the material which was partially decalcified in Müller's fluid by means of celloidin and frozen sections of undecalcified bones.

The Pathological Condition Produced in the Skeleton by the Administration of Diets Deficient in their Content of Fat-Soluble A and Phosphorus.—The diets described above (diets of lots 2667 and 2806) give rise to pathological pictures in the bone presenting a wide range of variation. At one extreme, the picture closely resembles that of rickets as that disease manifests itself in the human being, at the other extreme, osteoporosis.

The autopsy findings in the rats whose condition most closely resembled rickets, were as follows: The animals were undernourished and under-developed. Their coats were rough and uneven. Xerophthalmia was invariably present. The teeth were loose; the incisor teeth were easily fractured or extracted. In some animals spontaneous fracture of one of the incisors had occurred. The cranial bones were soft. The costochondral junctions were enlarged and the oval swellings formed were unusually long. In some rats there was moderate displacement inwards of the middle tier of costochondral junctions. In some animals a few fractures of the ribs marked by callous formation but without deformity of the costal arch were present. The spleen was atrophic in some animals, in others more or less enlarged. The thymus was atrophic. There was little or no fat. The long bones were straight and were not factured. The ends of the long bones were greatly enlarged. The femur and tibia were easily cut and gave little evidence of the presence of calcium salts until the knife had entered well into the shaft. Gross examination of the cut surface revealed a broad zone of a pale tissue, 2-4 mm. in width, lying between the epiphyseal cartilage and the shaft (the rachitic metaphysis). [161]

The epiphyseal cartilage in some animals was increased in width throughout the entire area of the section. It was more common, however, to find it of a normal width or a width not greatly exceeding the normal except at the periphery of the bone. At the periphery prolongations of cartilage extended diaphysealwards well into the metaphysis. Higher magnification showed the matrix of the cartilage to be unusually abundant in proportion to the numbers of cells present. The undifferentiated portion of epiphyseal cartilage was represented by a thin zone in which the cells appeared to be unusually far apart. The proliferative zone contained columns of cells aggregated in bullet-shaped masses, the points of which were directed toward the undifferentiated cartilage. Each bullet-shaped mass was separated from its fellows by a broad band of matrix (Fig. 3). As the metaphysis was approached, the cells of the proliferative zone, which were small and often flattened, with lense-shaped nuclei, grew larger and assumed a nearly spherical form, and at the same time underwent a progressive loss of affinity for the basic dyes. The nuclei became larger and the staining reaction approached more nearly that of the osteoid tissue in the metaphysis (Fig. 4). At the points of junction of the cartilage with the metaphysis the cartilage cells lost their columnar arrangement and became heaped together in disorderly masses. There was no evidence of calcium deposition in the zone of proliferative cartilage corresponding to the zone of provisional calcification in the normal bone, or else the calcification was fragmentary. The cartilage was invaded to a considerable extent by blood vessels from the marrow cavity, but did not appear to be greatly disorganized. The invading vessels for the most part were

[161] slender and appeared to advance, in some instances at least, along the strands of matrix between the columns of cells.

The metaphysis was composed chiefly of osteoid trabeculæ but also of blood vessels carrying with them marrow elements, tongue-like bands of cartilage cells of various thicknesses continuous with the epiphyseal cartilage and of islands of cartilage cells. The trabeculæ were broad. Many of them contained central cores of uncalcified cartilage and in almost all of them single cartilage cells could be identified or small groups in various stages of transition to a state indistinguishable from the cells of the osteoid itself. The trabeculæ were separated from each other by wide blood channels each of which was surrounded by a small amount of delicate connective tissue and a few marrow elements. The osteoblasts covering the trabeculæ were flattened, spread out like endothelial cells and had small nuclei. The osteoid of the trabeculæ was for the most part laid down in the form of lamellæ, but the lamellar arrangement could not always be made out. The bone corpuscles varied in size, but by far the majority were, to judge from their nuclei, small cells. The large prolongations of the epiphyseal cartilage into the metaphysis seemed to retain for the greater part of their extent the normal staining reaction to hæmatoxylin (Fig. 4). The smaller prolongations, those consisting of thin strands of cartilage cells, and the [162] terminal portions of the large prolongations just alluded to, seemed to lose their staining reaction almost entirely. The loss of the staining reaction appeared to occur in the neighborhood of the blood vessels and apparently depended on the intimacy of contact between the cartilage cells and the vascular elements. It was most marked in the strands or groups of cartilage cells which had become incorporated in the interior of the osteoid trabeculæ. As the cartilage cells came into close relationship with the marrow elements, they also appeared to undergo changes in their morphology. The cell bodies became smaller and took on a vesicular appearance. Around the cell body there developed a homogeneous material having the same staining reactions and outward appearance as the uncalcified material formed by the osteoblasts. In the osteoid trabeculæ removed some distance from the epiphyseal cartilage, where the change taking place in the cartilage cells appeared to be far advanced, the cartilage cells were so reduced in size that they could be distinguished from the osteoblasts only by their oval cell capsule and the vesicular appearance of the cytoplasm, or could be differentiated with the greatest difficulty or not at all. In some osteoid trabeculæ cartilage cells could be found in all stages of transition to osteoblasts.* The cartilage cells embedded in the osteoid trabeculæ seemed to be stimulated to reproductive [162] activity, for areas were seen where single cells had obviously given rise to from two to five daughter cells (Fig. 5). Cell division was apparently accomplished by amitosis. The cartilage cells composing the small islands in the metaphysis underwent the changes just enumerated. The cartilage cells composing the larger islands, however, retained their ordinary staining reactions except at the periphery where an abrupt change to osteoid took place. These large islands of cartilage cells appeared to be insulated by thin rims of osteoid. It is important to note that the tendency toward calcium deposition must have been very marked even in those animals that exhibited the pronounced changes of a rachitic nature just enumerated, for in the bones of the great majority of them linear deposits of lime salts were found which passed transversely across the metaphysis at right angles to the long axis of the bone. These deposits were sharply separated from the shaft of the bone, on the one hand, and from the proliferative zone of cartilage on the other. In some animals a single bone showed two or three of these evidences of abortive attempts at healing (Figs. 8 and 9). Such bones were striking in their resemblance to the bones of children showing the lesions of so-called " healing rickets."

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* The disappearance of the staining reaction of the cartilage cells and the morphological changes enumerated do not indicate a degenerative process. On the contrary, the existence of the cartilage cells appeared to be prolonged in a remarkable manner. As the

In the shaft of the bones of the animals showing extreme changes of the nature just described, the formation of osteoid tissue about the trabeculæ was markedly increased (Fig. 6). The small trabeculæ of calcified bone were completely surrounded by broad areas of osteoid and the periosteum was widely separated from the cortex by masses of osteoid tissue (Fig. 7). New bone formation in fibrous tissue was found occasionally beneath the periosteum in the region of the metaphysis. The osteoid tissue on the periosteal side of the cortex was traversed by numerous blood vessels. Here and there it was possible to find evidences of bone resorption. The evidences of resorptive activity in the metaphysis were very slight.

In animals at the other extreme the abnormality was of the nature of an osteoporosis. The epiphyseal cartilages were perhaps slightly irregular, but they were narrow. There was no evidence of abnormal persistence of the cartilage cells. Heavy continuous deposits of lime salts were present in the diaphyseal border of the cartilage, and this latter terminated abruptly in contact with the shaft. The marrow cavity was crowded with hæmatopoietic cells. The cortex was thin but completely calcified. In the medullary cavities of some bones

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cartilage was invaded by the capillaries, its cells became embedded in osteoid through the activity of the osteoblasts. In this envelopment of osteoid they persisted and became indistinguishable from the cells of the osteoid investment. Our experience would lead us to believe that the cartilage cells may undergo metaplasia into the cells of the osteoid.

[162] a few very thin, completely calcified trabeculæ were present, but in others the only bone to be found was the cortex of the shaft. In some of the rats showing these extreme osteoporotic changes the trabeculæ were invested with fibrous tissue and around them were to be found basophile cells with single nuclei and large granules. The evidences of resorptive activity in the trabeculæ were well marked. No osteoid was present anywhere. The gross *autopsy findings* in these animals were in accord with the picture which the microscope revealed in the sections. There were no deformities of the skeleton of any sort, but the bones were abnormally slender, a finding especially noticeable in the ribs, because of the contrast between the thin shaft and the costal cartilages. The latter appeared to be of normal size or even larger than normal. The bones gave evidence of the presence of calcium salts when an attempt was made to cut them; they were brittle and fragile, although there were no fractures to be found.

All stages of transition were found between the two extremes of the pathological process induced in the skeleton by the diets low in fat-soluble A and phosphorus, and by far the greater number of the animals examined may be said to have exhibited pathological changes of a mixed nature. In all these animals of the intermediary group the pathological picture presented had a rachitic background. The deposition of the calcium salts in the cartilages was irregular, and areas were found in the proliferative zone in which lime salt deposits were not present. The cartilages in most of the rats were extremely irregular, and in a considerable number of animals there was persistence of the cartilage with the formation of a thick proliferative zone (Fig. 10). In this thick proliferative zone large defects were noted due to invasion of the cartilage by blood vessels. In some of the animals, although calcification of the cartilage had occurred, prolongation of the cartilage was pronounced. Other animals showed cartilage cells in calcified as well as uncalcified portions undergoing the changes already alluded to which have been probably wrongly regarded as signs of degeneration. In almost all the rats showing these mixed lesions there was a narrow metaphysis or a partial metaphysis, *i. e.,* one extending part way across the bone. All the changes characteristic of rickets in the human being were present with the exception of the absence of calcium deposition in the proliferative cartilage. The number of trabeculæ in the shaft, however, was greatly reduced and, while even broad zones of osteoid were in evidence about the trabeculæ, there were many signs that the trabeculæ were in process of resorption and were being removed from the medullary cavity (Fig. 11). The gross changes in the skeleton consisted merely in varying degrees of enlargement of the costochondral junctions and the ends of the long bones of the extremities.

Experiments Designed to Show the Effects on the Skeleton of Diets Low in Their Content of Fat-Soluble A, But Having an Adequate Amount of Phosphorus [162]

Diets of Lots 3120 and 2815

Rolled oats	40.0
Gelatin	10.0
Salts (185)*	3.7
Dextrin	46.3

100 grams of this mixture contained 0.3150 gms. of calcium, and 0.5383 gms. of phosphorus.

NaCl	0.173	[163]
$MgSO_4$ (anhydr)	0.266	
$NaH_2PO_4 + H_2O$	0.347	
K_2HPO_4	0.954	
$CaH_4(PO_4)_2 + H_2O$	0.540	
Fe citrate	0.118	
Ca lactate	1.300	

Of this mixture 3.7 gms. were added to every 100 gms. of ration.

This diet, as is easily seen, contains protein of fairly satisfactory amount and quality. The properties of the mixture differ from those of the diets of Lots 2806 and 2667 in that there is contained a salt mixture having among other things the sodium, chlorine, calcium and phosphate ions in satisfactory amounts. The diets of Lots 2806 and 2667, however, lacked the phosphate ion from among the necessary supplements for the inorganic moiety of this diet. When young rats are restricted to this diet they soon fail to increase in weight and then slowly decline. They invariably develop xerophthalmia and die. This diet supplemented with some substance acting as a source of fat-soluble A induces growth and apparently fairly satisfactory nutrition over a considerable period.

The Pathological Conditions Produced in the Skeleton by Administration of the Diet Deficient in its Content of Fat-Soluble A, but Containing an Adequate Amount of Phosphorus

The eleven rats fed on this diet were all small and exceedingly mal-nourished. The eyes showed the characteristic xerophthalmia.

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* Composition of salt mixture 185. [162]

[163] The incisor teeth were fragile, loose in their sockets, and in some animals fractured. On removal of the skin there was a notable absence of fat. The thorax externally showed no deformity. On opening the thorax the arch of the ribs and cartilages was normal and there was no deformity or enlargement of the costo-chondral junctions. The shafts of the ribs appeared extremely thin, and the costal cartilages large as compared with them. No fractures of the ribs were present. The vertebral column was not bent. Both thymus and spleen were atrophic. The fore and hind legs were slender, but presented no deformities, fractures, or enlargements of the ends of the long bones. On section of the femur and tibia the resistance was greatly diminished, but the bones grated under the knife as the latter entered the shaft. On examination of the cut surface the cortex was found to be exceedingly thin. The marrow cavity extended close to or actually to the cartilage. There was either no sub-chondral zone of spongiosa, or an exceedingly thin one. The epiphyseal cartilage separating the large nucleus of ossification from the shaft appeared as a narrow band, the width of which was everywhere equal. By means of the binocular microscope there could be detected a continuous thin line of calcium deposit along the diaphyseal border of the cartilage.

On microscopic examination the results of gross examination were corroborated. The band of epiphyseal cartilage was found to be exceedingly narrow, and the columns of cartilage cells correspondingly short. Calcification of the proliferative zone lying in contact with the marrow cavity was complete. The character of the calcification corresponded closely to that found in the bones of animals whose growth is approaching completion, that is, each cell was completely enveloped by calcium deposit. The diaphyseal portion of the bone also had in some respects the appearance which characterizes the bones of animals whose growth has almost ceased, that is, there were few trabeculæ immediately adjacent to the cartilage; in some of the animals there were none (Fig. 12). When trabeculæ were present they were most numerous at the periphery, filling in the angles between the epiphyseal cartilage and the cortex. In the central portion of the bone in all the rats the marrow cavity was in immediate contact with the epiphyseal cartilage. The trabeculæ themselves were thin. In most of the rats they showed no osteoid borders anywhere. In some animals, however, the trabeculæ here and there were partially covered with borders of osteoid, some of which slightly exceeded the normal in thickness. The cortex was thin, completely calcified, and solid, *i. e.*, it did not contain spongy tissue. In the bones of the majority of the animals the signs of resorption were well marked. The trabeculæ were invested with layers of fibrous tissue, and in the periphery of the trabeculæ were countless numbers of rather large mononuclear cells conspicuous for the presence of basophilic granulations. These cells were thought to be connected with the resorptive process. In many trabeculæ holes and cavities could be found in which osteoblasts could be identified lying free, and in other places osteoblasts could be seen lying half in the calcified part of the trabeculæ and half in the osteoid border. Where osteoid borders were present, they appeared in the preparations stained with hæmotoxylin-eosin to be exceedingly pale, almost translucent, and the cell bodies of the osteoblasts in them, apparently strongly basophilic, were clearly defined in contrast. The osteoblasts themselves were large and in the trabeculæ appeared unusually close together. The nuclei were large and in those cells lying in the osteoid (where the cell bodies could be studied) were eccentrically placed in the cell body. The cell body was not round but irregularly oval. Near the nucleus at about the center of the cell was a large, round area of vacuolization about the size of the nucleus. It was by means of this area of vacuolization that the osteoblasts lying free in the holes in the trabeculæ or along the sides could be identified with such certainty. Many of the trabeculæ were covered with large numbers of osteoblasts. We are inclined to believe that the osteoid borders were caused not by growth but by removal of the lime salts, *i. e.*, halisteresis. The marrow, with the exceptions already noted, was normal. The basophilic cells which surrounded the trabeculæ were in no instance found in the marrow cavity.

Discussion

The first two diets used in these experiments (diets of lots 2667 and 2806) characterized by a deficiency in fat-soluble A and phosphorus, produced, as anticipated, xerophthalmia and changes in the skeleton. The xerophthalmia, as nearly as we could judge from gross inspection of the eyes, did not vary except in degree. The changes in the skeleton, however, exhibited a wide range of variation. In the animals at one extreme (in 13 rats) the ends of the long bones were characteristically large. The zone of proliferative cartilage in places was greatly broadened and continued in long processes toward the shaft. The cartilage cells in these processes and those elsewhere in contact with the marrow elements of the metaphysis exhibited the characteristic morphological changes and loss of staining properties so commonly seen in the rickets of human beings. The metaphysis itself was broad and composed, in addition to the vascular elements, almost entirely of osteoid trabeculæ. Calcification of the zone of the proliferative cartilage, corresponding to the provisional zone in the normal bone, was either entirely absent or very deficient. In

[163] almost all instances, however, there were broken linear deposits of calcium in the metaphysis, extending across it at right angles to the long axis of the bone. The pathological condition in these animals, showing the more extreme changes, exhibited all the fundamental characteristics of rickets, as it manifests itself in human beings, and may be said to have
[164] borne a strong resemblance to a certain form of rickets occurring in the human being, characterized pathologically by linear zones of lime salt deposition in the metaphysis, indicating alternate healing and exacerbation.

In the animals at the other extreme (in 9 rats) the pathological condition corresponded to what is ordinarily included under the term osteoporosis. The epiphyseal cartilage was reduced to a narrow band. The transition between cartilage and shaft was abrupt. Calcification of the cartilage was regular and complete. The few thin trabeculæ were free from osteoid, and resorptive phenomena were abundantly in evidence. The pathological condition of the bones exhibited by the rats at this extreme of the series showed no evidences of rickets whatsoever, but on the contrary complete calcification of all elements both cartilage and trabeculæ.

The majority of the animals on these two faulty diets (27 rats), however, showed pathological conditions in the skeleton intermediary between the two extremes just briefly outlined. The trabeculæ were bordered with osteoid, not the thin border present in growing young animals, but broad zones comparable to those seen in the advanced rickets of children or even exceeding them in thickness. In all there was irregularity and broadening of the epiphyseal cartilage and defects in its calcification, in some instances most extensive, in others small. Some animals showed short metaphyses or partial metaphyses, in which were columns or masses of cartilage in an uncalcified or in an incompletely calcified state, displaying the morphological changes and alterations in staining reactions previously enumerated. The number of the trabeculæ were few, and in the trabeculæ, in particular those close to the cartilage, there were present abundant evidences of resorptive activity. The pathological conditions displayed in the bones of the rats showing these intermediary changes bore a marked resemblance to the not very advanced rachitis of human beings, [164] when the healing process has been well established or has progressed far toward completion. An anomalous condition was present characterized by abundant calcium deposits in the cartilage on the one hand, and borders of the trabeculæ still uncalcified on the other, a well recognized and characteristic intermediary stage in the healing of the rachitis in human beings.

It is not possible to explain why all the rats fed the diets deficient in fat-soluble A and phosphorus failed to develop exactly the same changes in the bone. It is possible that the diets in question were not absolutely constant in their composition, not as regards a deficiency in fat-soluble A—for all the rats without exception developed xerophthalmia—but as regards some other substance. The stock supplies of flaxseed meal and rolled oats were replenished during the course of the experiments several times, and may have been derived from different sources.* A reason for suspecting that variations in the composition of the diets may have had some influence is that the animals which most consistently developed the marked rickets-like changes were the first animals of the series (those of the experiments completed before May 19, 1920). Another explanation, however, must be considered. As already pointed out, there were noted in all the animals, even in those showing the most marked rachitic-like changes, evidences of a strong tendency toward healing, manifesting itself in the deposition of calcium salts in the cartilage. In a large proportion of the animals the healing process, as measured by the extent of calcium deposition in the cartilage, appeared to be far advanced. If the composition of the diets was essentially constant, it is necessary to suppose that the abnormal conditions in the body induced by them, under which lime salts could *not* be taken up by the cartilage and the bone, were not very far removed from other conditions more nearly approaching the normal under which lime salts

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* The content of the diets in calcium and phosphorus was calculated from the average composition of rolled oats and flaxseed meal, as compiled by Forbes, Sherman and others, and from the composition of the salt mixtures employed.

[164] *could* be taken up by the cartilage and the bone, and that the former state could be transformed into the latter by slight changes in the animals' metabolism. In other words, the particular abnormal equilibrium of the forces concerned with calcification and ossification produced by the faulty diets was unstable. With the rapid decline in the nutrition of the animals occurring during the latter part of the experiments and the corresponding loss in weight, a complete cessation, or at least a great retardation in the rate of growth of the skeleton must have occurred. There must have come about, therefore, a diminished requirement for those substances essential to ossification and calcification, which were insufficiently supplied in the diets. Moreover, it seems probable that a supply of those substances may have been liberated from the tissues of the animals themselves, for example, as the result of the resorptive processes in operation in the bones. From a theoretical standpoint, therefore, it seems possible to think that the processes in operation in the animal leading to the development of the state of extreme malnutrition may have been instrumental in restoring to the organism conditions under which calcium deposition in the skeleton again became possible. For the development of rickets growth is necessary. If the deficiencies in the diet are of such a nature as to render growth of the skeleton impossible, rickets cannot develop. If growth were brought to an end in an animal already rendered rachitic through the administration of faulty diets, there is reason to expect that the rachitic lesion would disappear and a condition of osteoporosis develop. Whatever may have been the cause of the development of pathological changes in the skeleton, from the morphological standpoint, apparently so far removed from each other, there can be no doubt that the osteoporosis exhibited by some of the animals of the series was closely related to the healing process in evidence in the others, and represented merely the healing process in its completed state.

The pathological conditions produced in the rat by the diet low in fat-soluble A but containing a complete salt mixture (the diet of Lots 3120 and 2185) were absolutely constant and offered no difficulties of interpretation. In the entire series of eleven animals on this diet the band of proliferative cartilage was narrow, the junction between cartilage and shaft abrupt and even, the calcification of the cartilage heavy and everywhere complete, the trabeculæ thin; osteoid was not present at all, or was not in greater amount than could be accounted for by the resorptive processes, obviously in operation. The pathological condition induced in the group of rats fed this diet, deficient fat-soluble A, but containing the adequate amount of phosphorus, therefore, bore no resemblance whatsoever to rickets. It showed the typical picture of osteoporosis. [165]

The experiments recorded leave no doubt that the two diets having the known deficiencies in fat-soluble A and phosphate may produce in the bone of the rat changes having fundamental resemblance to the pathological changes in the bone which characterize rickets in the human being. At the same time they indicate that the addition of a salt mixture relatively high in its content of the phosphate ion completely prevents the development in the bone of the rat of changes resembling rickets, though it fails to exercise any preventive influence on the development of the xerophthalmia. It seems possible to infer from our experiments, therefore, that the development of rachitic-like changes in the rat induced by diets deficient in fat-soluble A and the phosphate ion can be prevented by the addition to the diet of phosphate-containing salts; and that conversely, for rachitic changes to develop in the presence of a deficiency of fat-soluble A in the diet, the diet in other respects being optimal, a low content of the phosphate ion is essential. The experiments indicate further that in the production of rickets-like conditions in the rat, fat-soluble A cannot be the sole factor concerned. Since the variable in the diets used in these experiments was certain phosphate-containing salts, it might seem possible to infer that it is necessary to consider only the inorganic content of the diet as active, and that a fat-soluble organic factor might be excluded from consideration. Even if it were granted that the induction of the rickets-like changes in the bone results from a disturbance in the level of the body's phosphate, it does not by any means follow that the inorganic ions are the only factors involved. Recent experiments in feeding cod-liver oil, which is known to be high in fat-soluble A but free from phosphate, to rats already rendered rachitic by means of the diet,

[165] have proven that an organic substance (cod-liver oil) may exert a powerful influence in causing lime salts to be deposited in the cartilage.[3] Studies on the effects of feeding cod-liver oil to rachitic children have led to similar conclusions.[4] Moreover, Howland and Kramer * have shown that the blood phosphate is low in the blood plasma of rachitic children, and that the administration of cod-liver oil causes a marked rise. It seems not unlikely that a similar result may be accomplished in rats by feeding a diet high in phosphate, and from present knowledge it seems reasonable to suppose that, while the calcification of the bones of children and animals fed diets high in their content of phosphate may be dependent on the elevation of the phosphate level in the blood, the phosphate curve may also be affected by the amount of the organic factor available for the body needs.

Finally, our experiments show beyond doubt that the addition of certain phosphorus-containing salts to the diet low in fat-soluble A in no wise prevents the development of xerophthalmia and indicates, as clinical observation has made it necessary to infer, that xerophthalmia and rickets cannot possibly have an identical etiology.

Conclusions

1. The two diets which were low in their content of fat-soluble A and phosphorus (diets of lots 2667 and 2806) produced in the majority of the young rats placed upon them pathological conditions of the skeleton having a fundamental resemblance to rickets. The pathological conditions produced are not identical, however, with that disease as it usually manifests itself in the human being.

2. The chief difference consisted in the presence of scattered or irregular deposits of calcium salts in the cartilage and metaphysis. The pictures bore a marked resemblance to those seen in rachitic children in whose bones incomplete healing has taken place.

3. When the deficiency in phosphorus is compensated for by the addition of a complete salt mixture containing the phos-

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* Personal communication.

phate ion (diet of Lots 3120 and 2815), the deficiency in fat-soluble A still existing, no pathological changes of a rachitic nature developed. The addition of the phosphate ion to the diets deficient in it and in the organic factor prevented, therefore, the development of any changes of a rickets-like nature. [165]

4. The experiments reported are not sufficiently numerous or comprehensive to permit of generalizations concerning the effects of a deficiencies of the fat-soluble A and phosphorus or of the fat-soluble A alone. The implication of the experiments is, however, plain.

(a) The phosphate ion in the diet may be a determining influence for or against the development of rickets.

(b) If the phosphate content of the diet is sufficiently high a deficiency of fat-soluble A cannot cause rickets-like changes in the skeleton.

(c) A deficiency in fat-soluble A cannot be the sole cause of rickets. Conversely, it is necessary that the diet be low in its content of phosphorus, all other factors, except fat-soluble A, being optimal for rickets-like conditions to develop.

5. Since the addition of the phosphate ion to the diet prevented the development of the rickets-like changes in the skeleton, but had no effect in preventing xerophthalmia, it seems permissible to infer that xerophthalmia and rickets do not have an identical etiology.

6. The above results do not in our opinion exclude the fat-soluble A from consideration as an etiological factor in the production of rickets and kindred diseases, since the level of the blood phosphate is, in all probability, determined in part by the amount of the fat-soluble A available for the needs of the organism.

BIBLIOGRAPHY

1. Mellanby, E.: The Lancet, March 15, 1919.

2. McCollum, E. V., Simmonds, Nina, Parsons, H. T., Shipley, P. G., and Park, E. A.: Jour. Biol. Chem., 1921, XLV, 333.

3. Shipley, P. G., Park, E. A., McCollum, E. V., Simmonds, Nina, and Parsons, H. T.: Jour. Biol. Chem., 1921, XLV, 343. [166]

4. Howland, John, and Park, E. A.: Arch. of Pediatrics, 1920, XXXVII, 411.

DESCRIPTION OF PLATES

FIG. 1.—Microphotograph showing advanced rickets-like changes in the lower end of the left femur. The picture shows the relative positions of the epiphyseal nucleus (e. n.), cartilage (c), the metaphysis (Met.) and the diaphysis (d) of the bone. The epiphyseal cartilage is persistent at the periphery of the bone.

FIG. 2.—Microphotograph of the lower end of the left femur showing marked rickets-like condition.

FIG. 3.—High power photograph of the epiphyseal cartilage—extreme rickets-like lesion showing the arrangement of the cartilage cells in the matrix.

FIG. 4.—Microphotograph of the epiphyseal cartilage from a rat showing a marked rickets-like condition, to show persistence of the cartilage cells and the degenerative changes in them.

FIG. 5.—This picture shows the cell division in cartilage cells which are in contact with tissues of the metaphysis. The cell capsule (c) contains four daughter cells which have arisen by the division of the cartilage corpuscle which originally occupied the capsule.

FIG. 6.—Trabeculæ in the medullary cavity of a rickets-like bone. The small spicule (o) which has undergone calcification is surrounded by a broad zone of osteoid tissue (o. s.).

FIG. 7.—This picture shows the sub-periosteal hyperplasia of the osteoid tissue—the so-called rachitic periostitis.

FIG. 8.—An abortive attempt at healing an extreme rickets-like lesion is recorded in the metaphysis of the affected bone by the linear deposit (1.) of calcium salt.

FIG. 9.—Microphotograph of a bone in which the metaphysis showed traces of three periods of calcium deposition, which are in all cases incomplete or have been partially absorbed.

FIG. 10.—This picture from a section of a bone which was in the transitional stage between the rickets-like condition and osteoporosis shows irregularity and prolongation of the epiphyseal cartilage, and its irregular calcification (cal). Broad zones of osteoid tissue (os) still persist about the trabeculæ.

FIG. 11.—The trabeculæ shown in this figure are further along in the process of absorption than those shown in Fig. 10. Calcification of the cartilage is incomplete in this bone. Osteoid tissue (os) is still present in the diaphysis.

FIG. 12.—Microphotograph showing the cartilage and part of the medullary canal of a bone from a rat maintained on a diet which included a complete salt mixture (185) high in phosphorus. This bone was extremely osteoporotic. The proliferative zone of cartilage (pz) is narrow and was completely and heavily calcified, as was the cortex. There were no trabeculæ in the medullary cavity, and no traces of osteoid tissue to be found in the diaphysis.

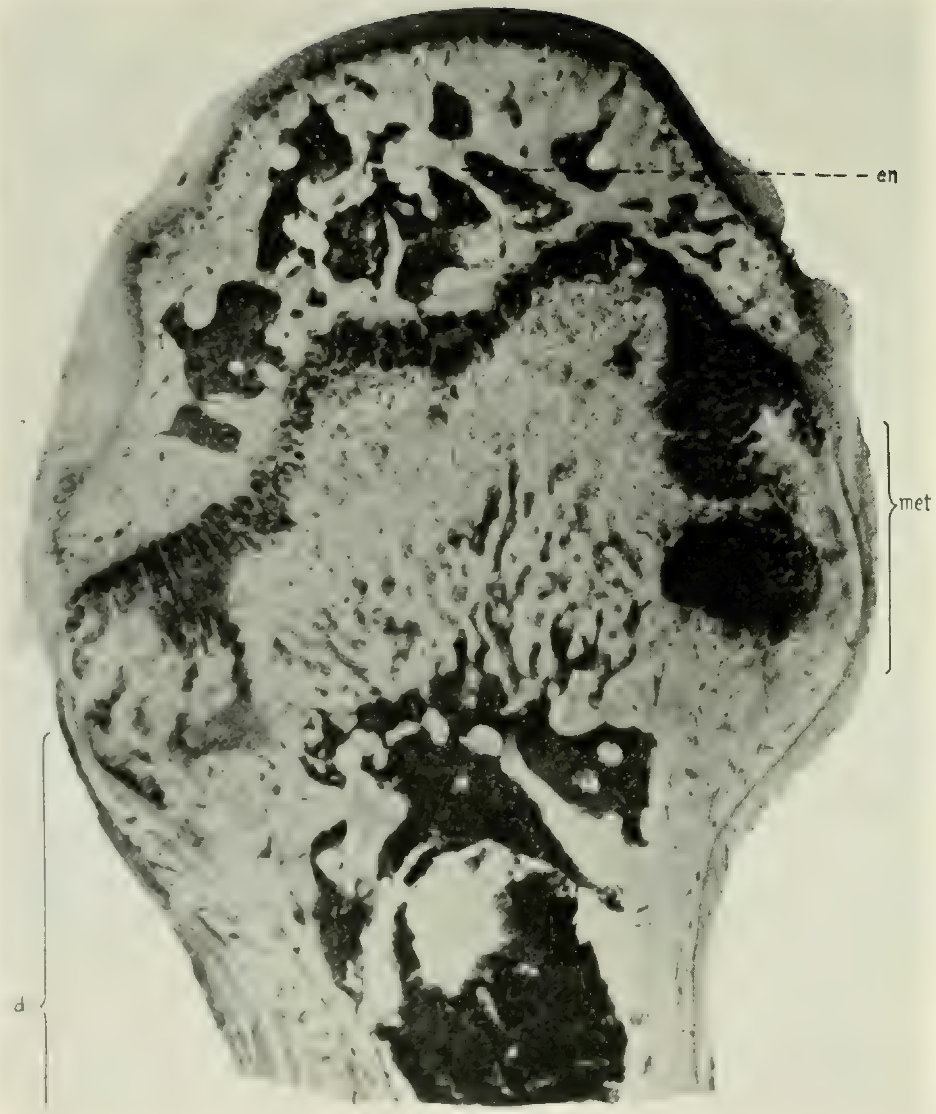


FIG. 1.

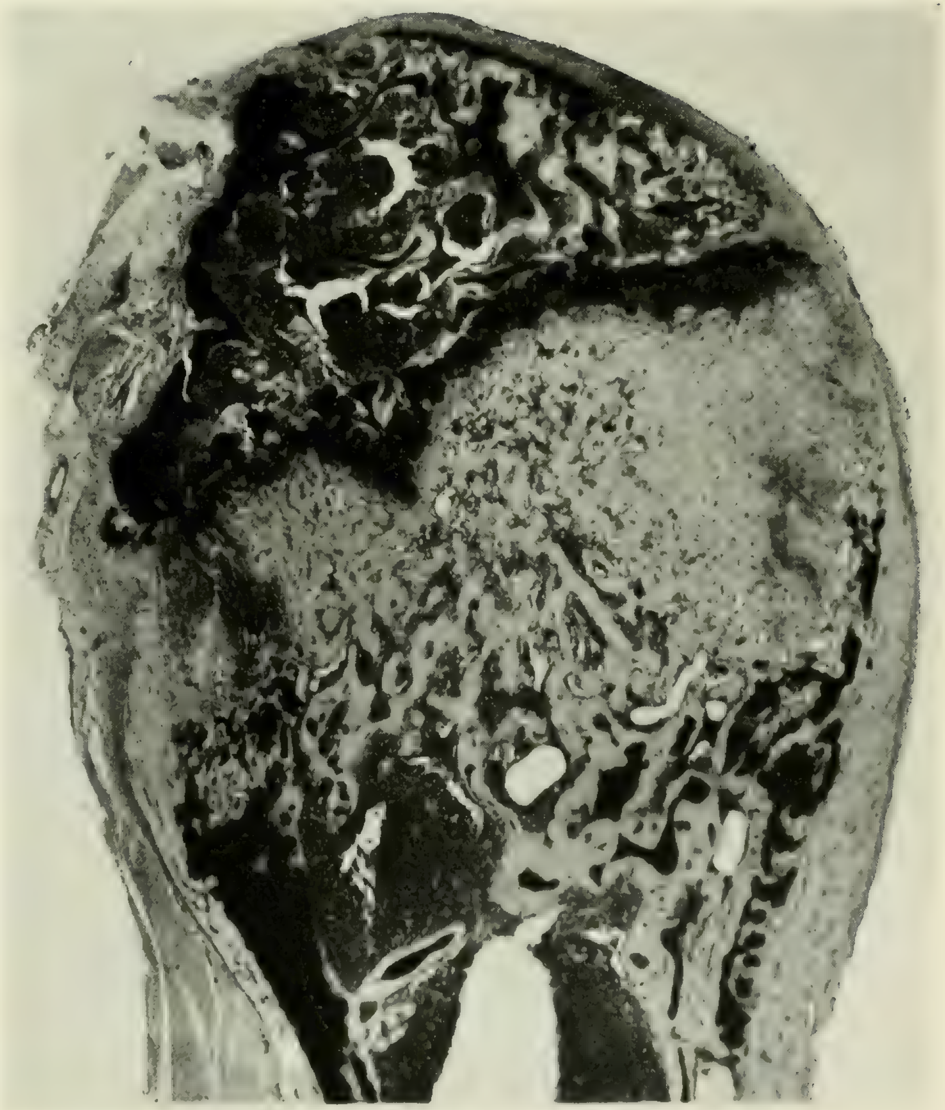
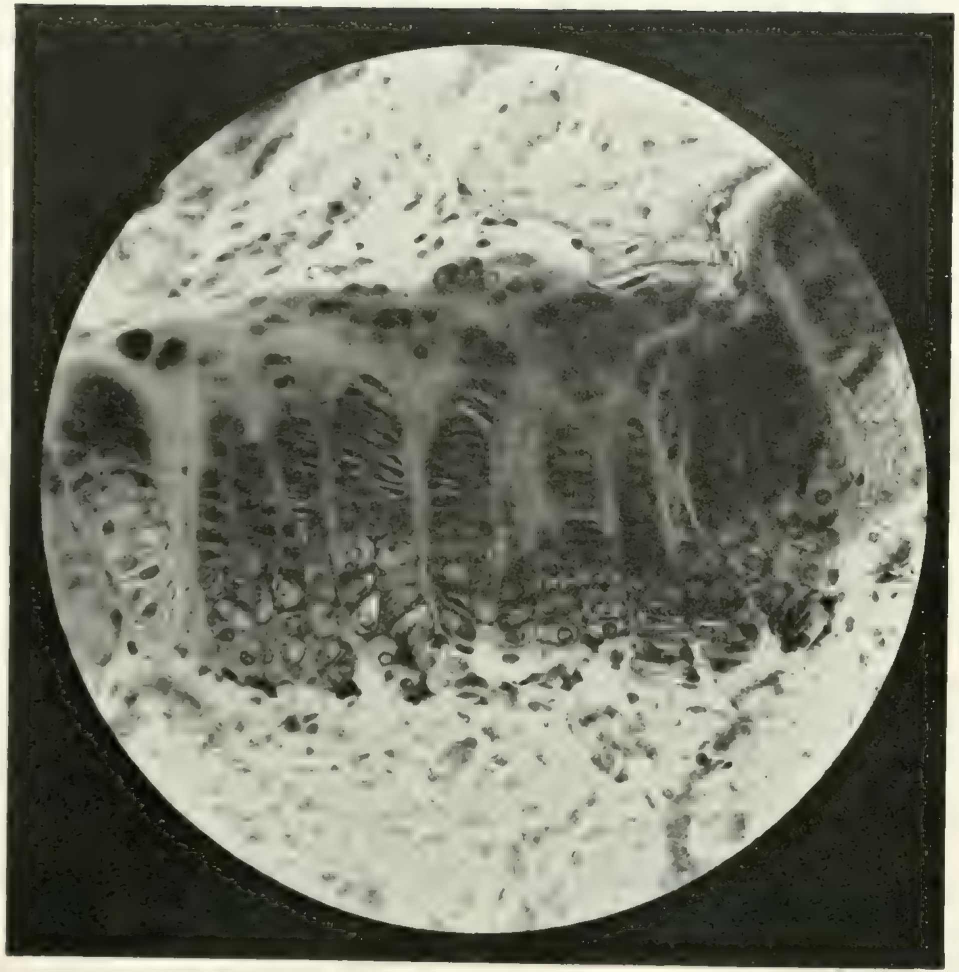


FIG. 2.



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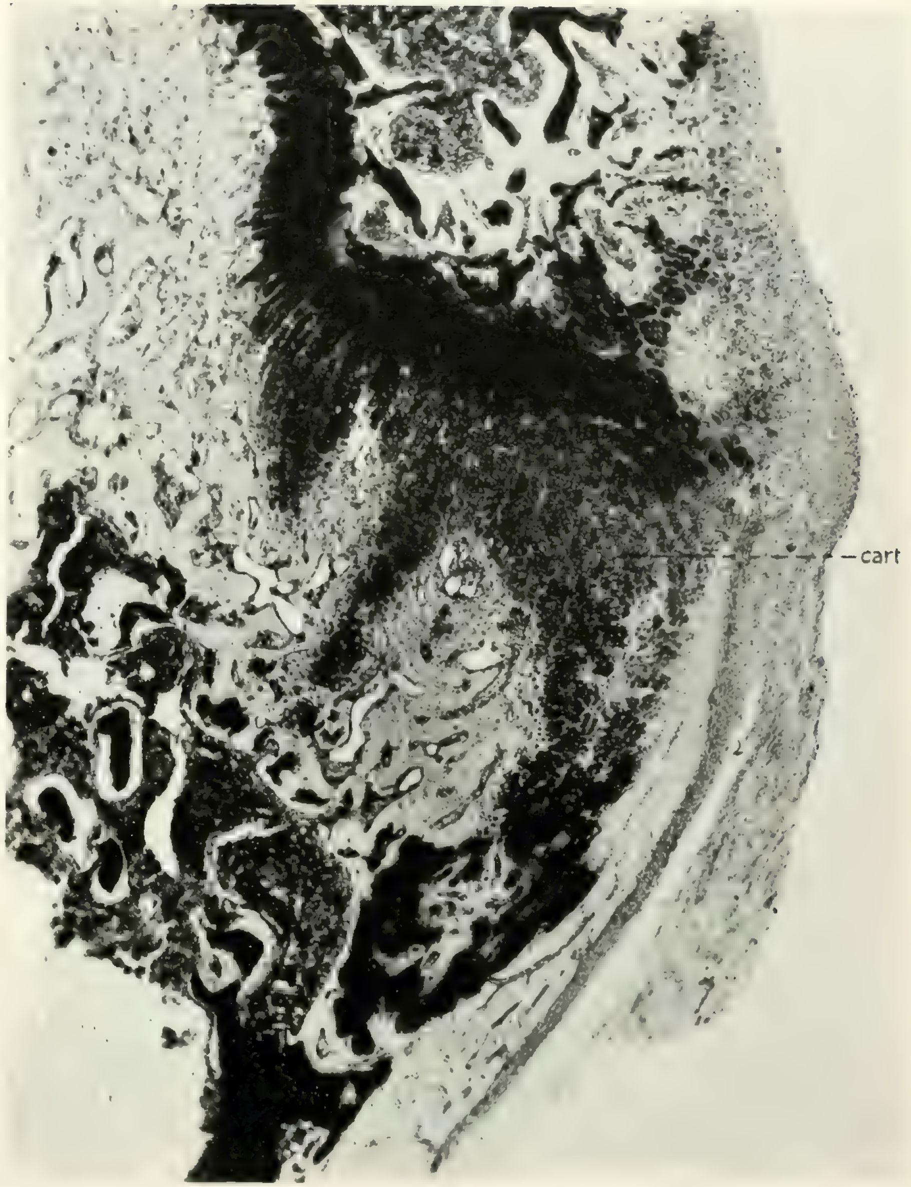

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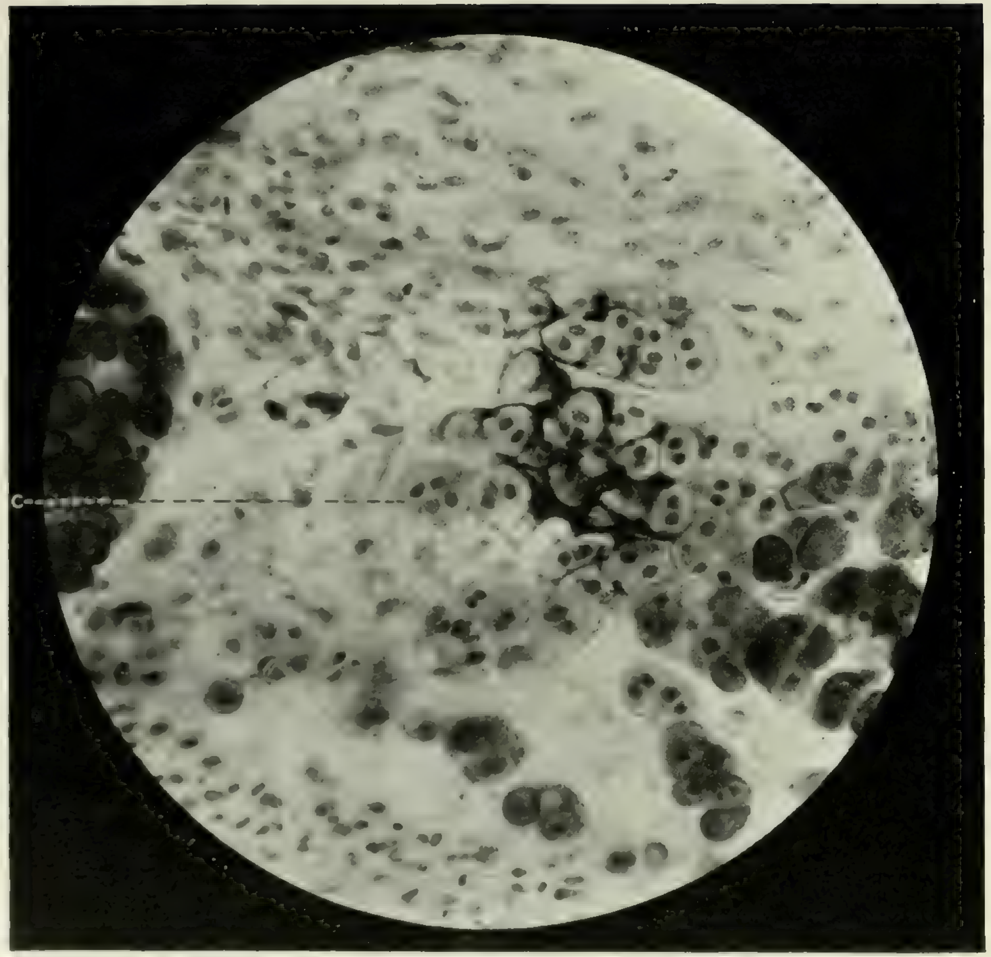

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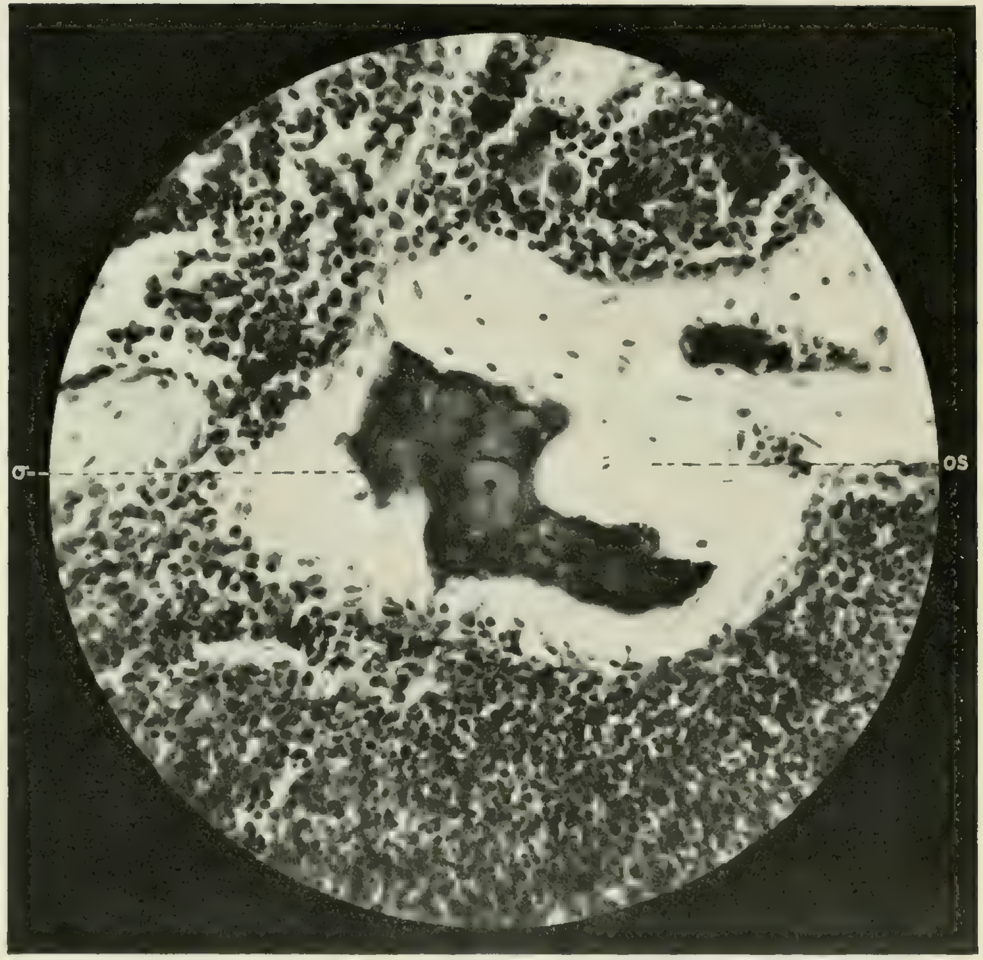

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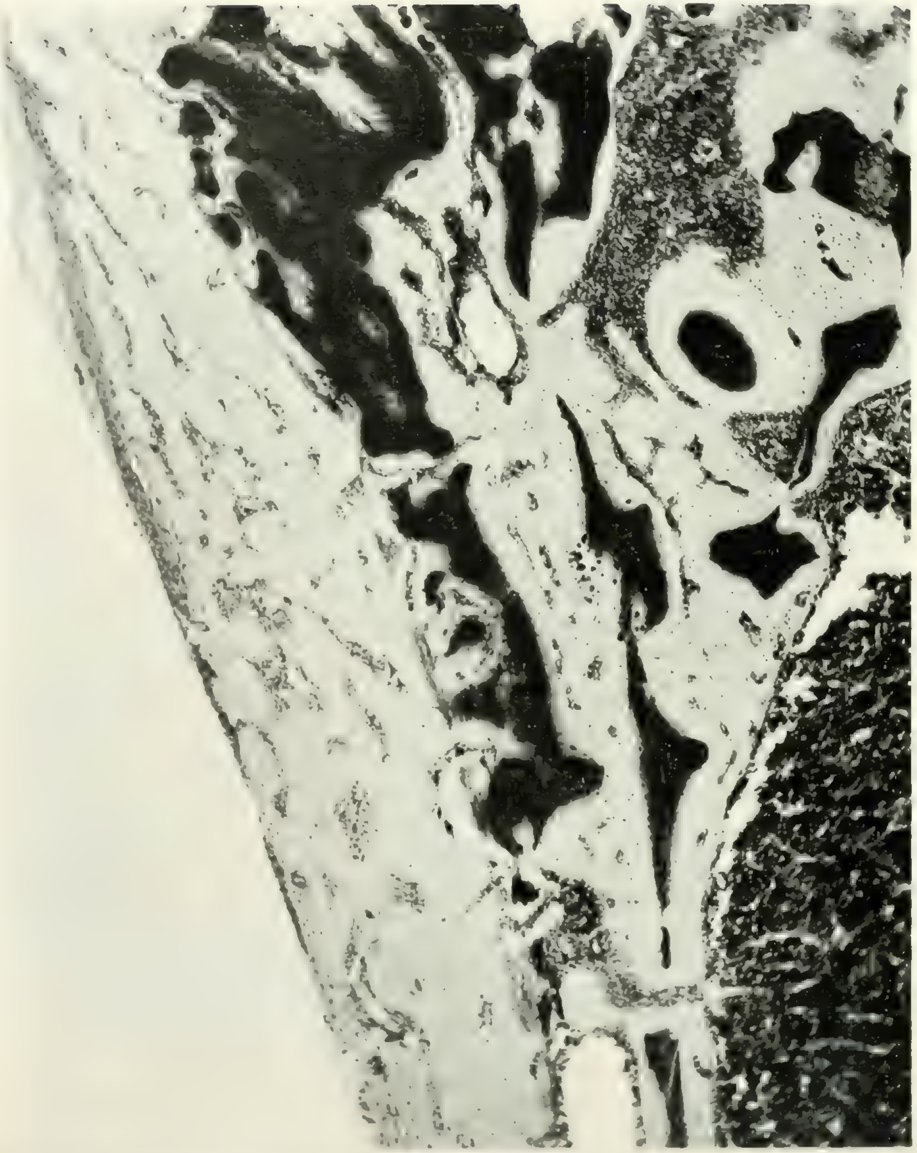

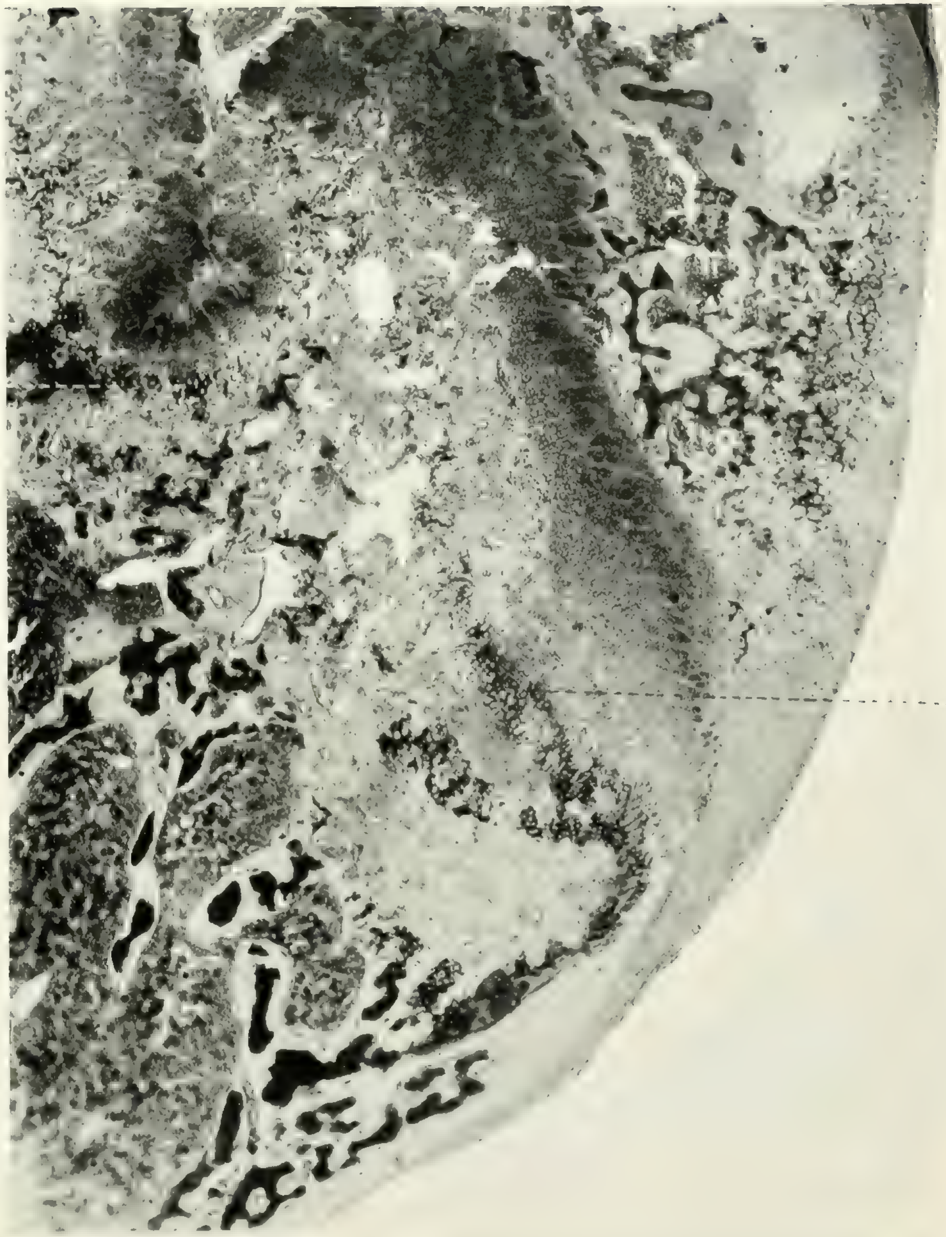

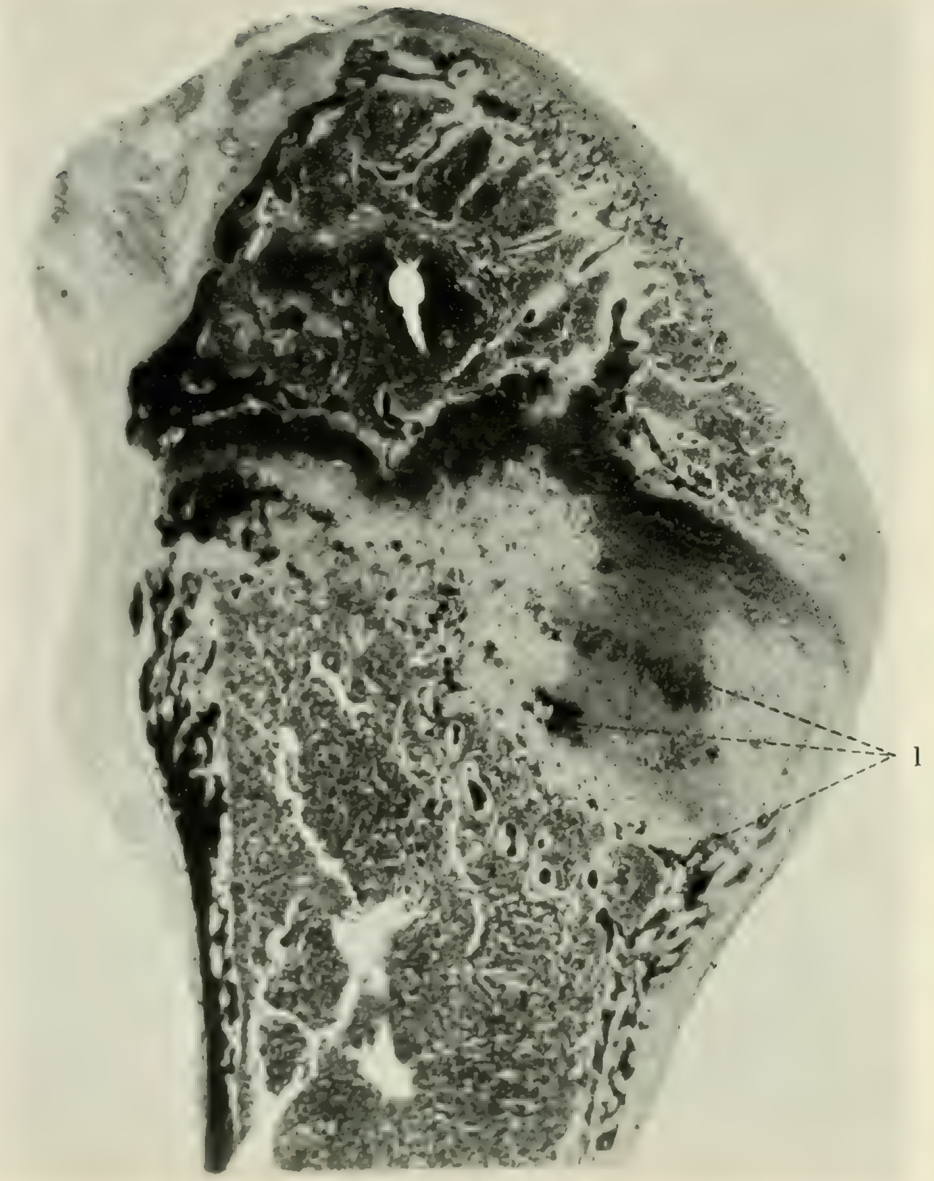

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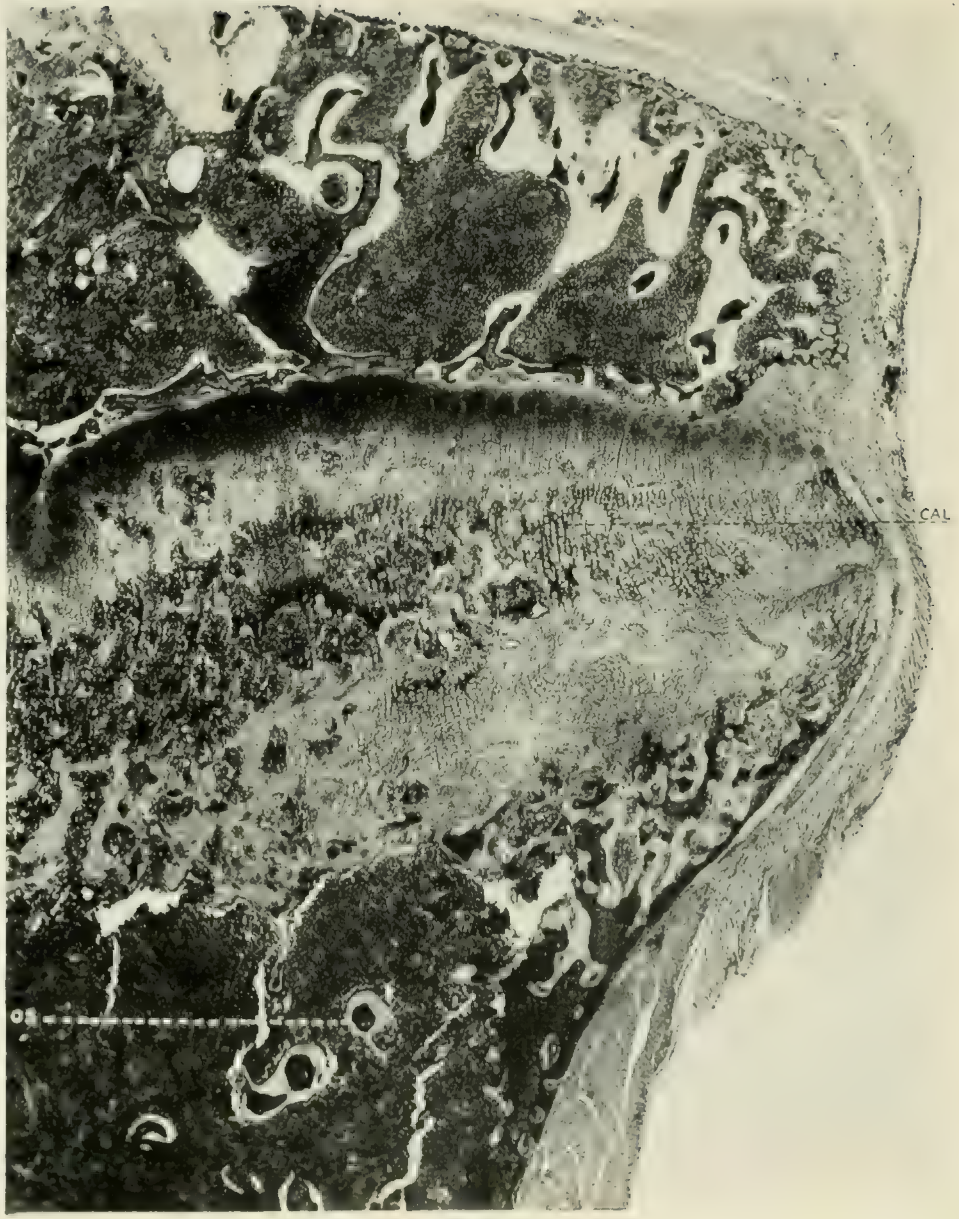

FIG. 10.

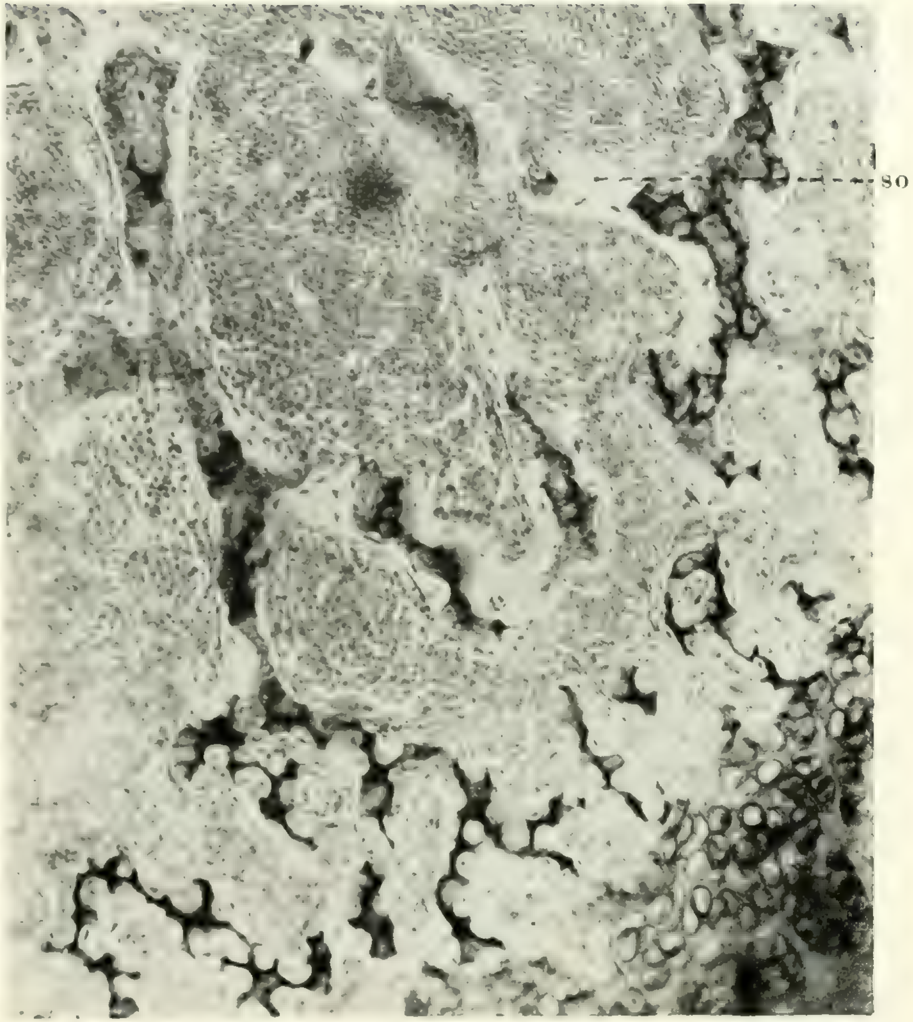

FIG. 11.

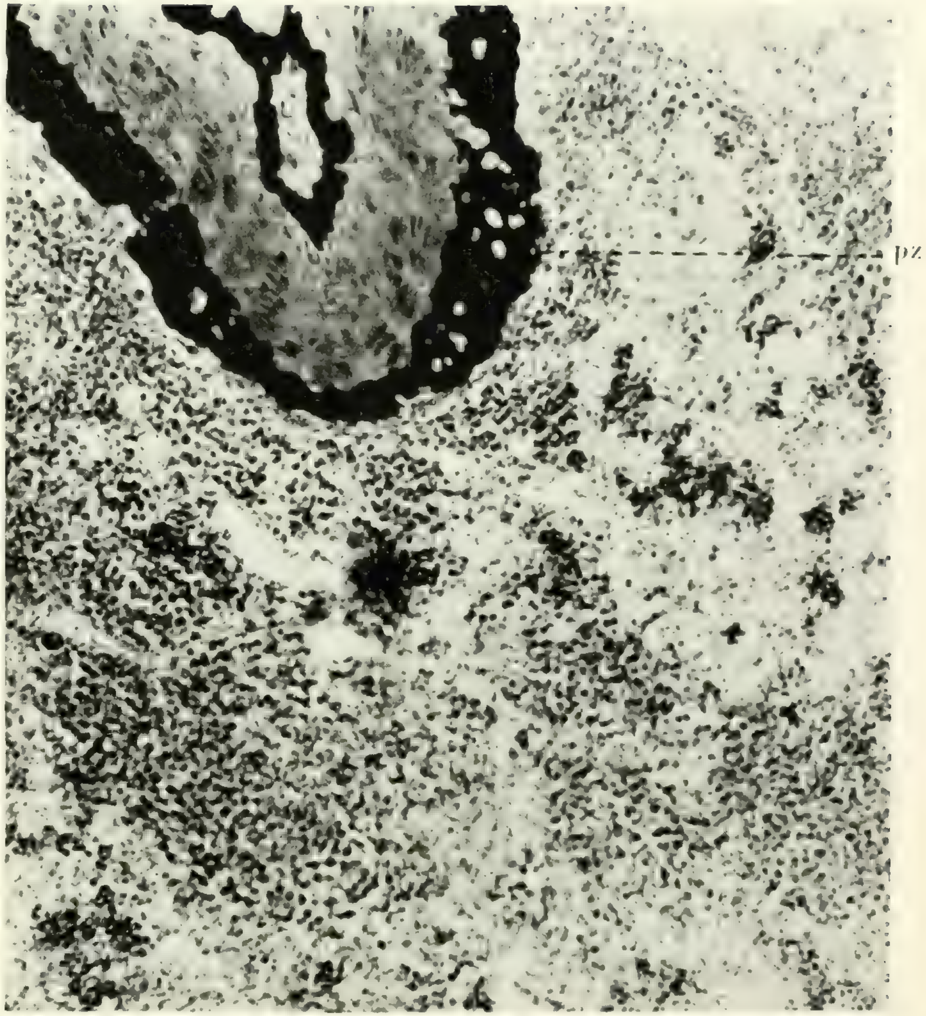

FIG. 12.

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THE ANTISCORBUTIC REQUIREMENT OF THE PRAIRIE DOG.

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Plates 2 and 3.

(Received for publication, September 27, 1920.)

In view of the fact that it is well demonstrated that certain species of mammals, as man, monkey, and guinea pig, cannot be adequately nourished on a diet which fails to supply a sufficient amount of the antiscorbutic substance (water-soluble C), whereas the rat can be nourished in a manner which at least closely approximates the normal during a long period without more than possible traces of antiscorbutic substance, it is of interest to examine other species in order to find how general among mammals is their susceptibility to scurvy.

We have, for example, seen young rats grow from weights of 40 to 60 gm. to essentially the full adult size on a diet of purified protein, dextrin, butter fat, a salt mixture, and agar-agar, supplemented with an alcoholic extract of 10 gm. of wheat germ per 100 gm. of ration to furnish the factor water-soluble B. One group of such rats was but slightly inferior in appearance after 15 months on this diet. The possibility is not excluded that these animals received a small amount of antiscorbutic substance from the wheat embryo extract, but their diet was one which would not have supplied sufficient antiscorbutic factor to prevent prompt development of scurvy in a guinea pig.

In the preceding paper Parsons[1] has discussed the problem of the source of the antiscorbutic factor in the livers of rats which were fed during long periods on diets which failed to afford any demonstrable protection against scurvy in a guinea pig. In the present communication we desire to describe an experiment which shows that another rodent, the western prairie dog (*Cynomys ludovicianus*), behaves like the rat rather than the guinea pig. The prairie dog is capable of growing during several months and remaining free from any symptoms of scurvy on a diet which utterly fails to protect the guinea pig against this disease.

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[1] Parsons, H. T., *J. Biol. Chem.*, 1920, xliv, 587.

In January, 1918, seven prairie dogs were secured from New Mexico through the courtesy of the Bureau of Animal Industry, United States Department of Agriculture. They were, for want of better facilities, kept in laboratory cages in a heated building. Two litters of young were secured, one of which was lost through accidental injuries to the mother. Two young from another litter were successfully weaned. These were employed as subjects in the experiment described in this paper. One died from digestive disturbances soon after the beginning of the experiment.

Our purpose in using these animals for this study was to compare them with guinea pigs in respect to their susceptibility to scurvy. We sought young prairie dogs for this study because, like the rat and guinea pigs, they are rodents, and of approximately the same size as the guinea pig. Furthermore, the diet of the prairie dog, at least during a large part of the year, consists of succulent grass rich in antiscorbutic substance.

The animal which grew to maturity, and the growth curve of which is presented in Chart 1, weighed 18 gm. when 1 day old, and at the age of 62 days weighed 169 gm. At this age (Period 2) it was placed on a modification of the soy bean flour diet found by Cohen and Mendel[2] to be satisfactory for inducing growth in young guinea pigs when supplemented with a suitable source of the antiscorbutic substance, but incapable without such addition of protecting the animals against the prompt development of scurvy. The diet used was essentially like that of Cohen and Mendel, except that it was poorer in antiscorbutic substance. They included in their diet sufficient rich Jersey milk to furnish 5 per cent butter fat. We replaced the milk in our diet by 5 per cent purified butter fat and soy bean flour.

Although this diet would lead to the development of scurvy in the guinea pig in 2 to 3 weeks, the young prairie dog in our

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[2] Cohen, B., and Mendel, L. B., *J. Biol. Chem.*, 1918, xxxv, 425.

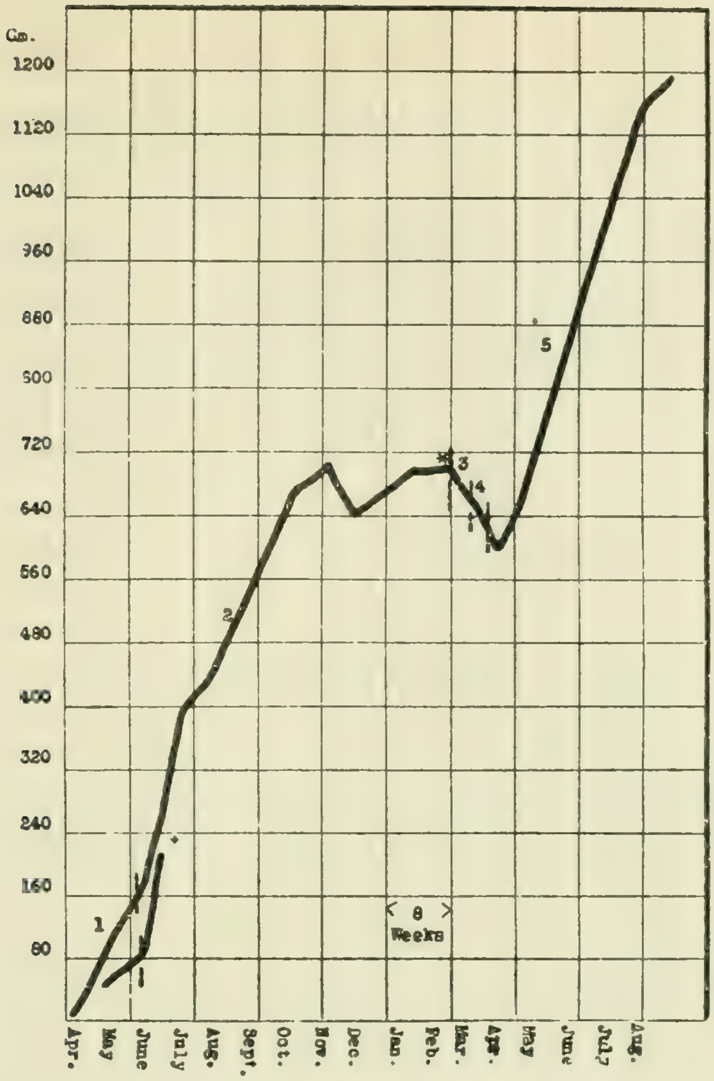

CHART 1. The long growth curve in the chart shows the history of a young prairie dog. Period 1 represents the period of nursing, to independence in diet. In Period 2 the animal was fed a diet consisting of cooked soy bean meal 84.0, sodium chloride 3.0, calcium lactate 3.0, dried yeast 3.0, butter fat 5.0, and filter paper 2.0 per cent. This diet contains too little antiscorbutic substance to be demonstrated with a guinea pig. On this diet the animal grew during 6 months from a weight of 169 to 690 gm. After this time there was very little growth on this diet. There is the possibility that the hibernating instinct was in part responsible for the cessation of growth which occurred about December 1.

The animal was photographed at the point marked by an asterisk. In order to determine whether the cessation of growth was due to lack of the antiscorbutic factor in the food, 7 to 9 gm. of peeled orange were given daily during 3 weeks, but the animal declined 40 gm. in weight during the period. In Period 4 the orange was omitted and 1 per cent cod liver oil was introduced into the diet. This modification did not arrest decline. In Period 5 the diet was changed to a mixture of grains and dried buttermilk, and after 4 days 5 gm. of green grass were given every few days. On this diet the prairie dog began promptly to gain in weight and continued to grow. During the following 6 months the animal grew from 604 to 1,195 gm.

The behavior of the animal in Period 2 on a scorbutic diet shows that this species is able to grow rapidly from nine to twelve times the interval necessary for the development of scurvy in the guinea pig on the same diet. At no time during the experiment did the prairie dog manifest any symptoms of scurvy.

experiment grew well on it during a period of 6 months. At the end of this time it showed no signs of scurvy but became lethargic and had a poor appetite. This was probably due in part at least to its hibernating instinct, for the cessation of growth happened about December 1. By March 1, however, the animal was awake and active, but did not resume growth. In order to discover whether this could be due to the lack of the antiscorbutic substance it was given 7 to 9 gm. daily of peeled orange. It did not at this time show any symptoms of scurvy. It declined about 40 gm. in weight during 3 weeks while receiving the orange, which seemed sufficient evidence that the decline was not due to the lack of this dietary factor. The orange was then discontinued, and 1 per cent of cod liver oil was included in the diet during the next 12 days, but this change did not arrest the decline. The animal was then changed to a ration consisting of mixed grains and dried buttermilk, and was given small amounts of green grass at intervals of a few days. It began to increase in weight within a week, and at the age of nearly 19 months weighs 1,195 gm., whereas it weighed 700 gm. at its highest point reached while fed the scorbutic diet. At the time of writing this paper (September 21) the animal is again beginning to appear drowsy and takes less than the usual amount of food.

The following explanations might be brought forward to account for the arrest in growth about December 1: (1) Lack of antiscorbutic substance; (2) instinct to hibernate at this season of the year; and (3) some other fault in the diet.

The first of these possible explanations seems satisfactorily disposed of by the failure of the animal to respond to 3 weeks feeding of orange juice.

Givens and Cohen[3] found a diet similar to that of Cohen and Mendel, consisting of soy bean flour 76.3; milk solids 11.4; dried yeast 2.9; paper 2.9; calcium lactate 2.9; sodium chloride 2.9 per cent, to be satisfactory for the growth of rats to maturity, but these rats failed to rear the young which were born. There can be no doubt that the food supply of our prairie dog, which was similar to their diet except that about 7 to 8 per cent of the nonfat solids of the milk was replaced by soy bean flour, was inferior in its nutritive value to theirs. This substitution was made for the purpose of eliminating a known source of antiscorbutic substance.

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[3] Givens, M. H., and Cohen, B., *J. Biol. Chem.*, 1918, xxxvi, 127.

The record of our prairie dog is of interest because it shows that this species is capable of growing for a period of 6 months on a scorbutic diet. This is equivalent to nine to twelve times the interval that is necessary for the development of scurvy in the guinea pig.

EXPLANATION OF PLATES.

Plate 2.

Figs. 1 and 2. The photographs were taken February 29, 1920. The animal had then been on the scorbutic diet 263 days. About December 1 it ceased to grow, but remained in good condition and at its maximum weight up to the time of taking these pictures. These photographs, therefore, show the excellent nutrition of the animal about the time when orange juice was introduced in the diet.

Plate 3.

Figs. 3 and 4. These photographs were taken September 19, 1920, after the animal had been on a mixed diet containing small amounts of grass for 153 days. Fig. 4 shows a marked accumulation of fat. This is probably sufficient to account for most of the gain in weight which followed the change from the scorbutic diet to the mixed diet containing the grass.

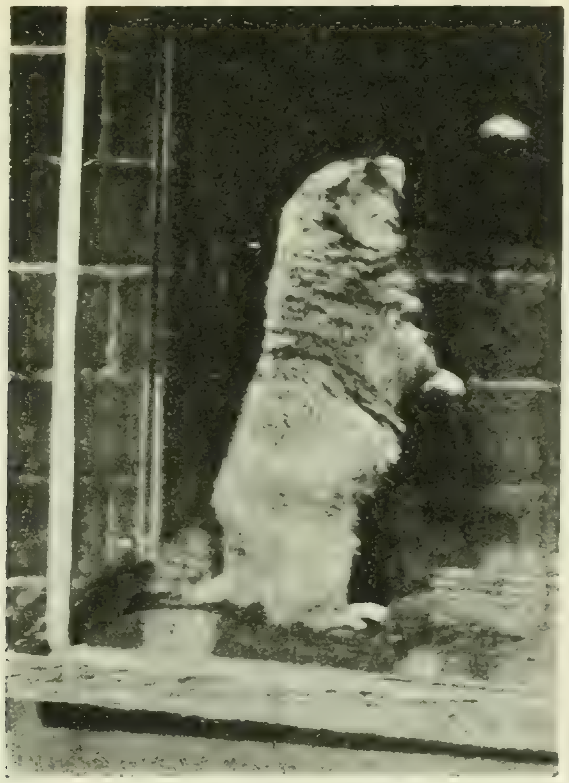

Fig. 1.

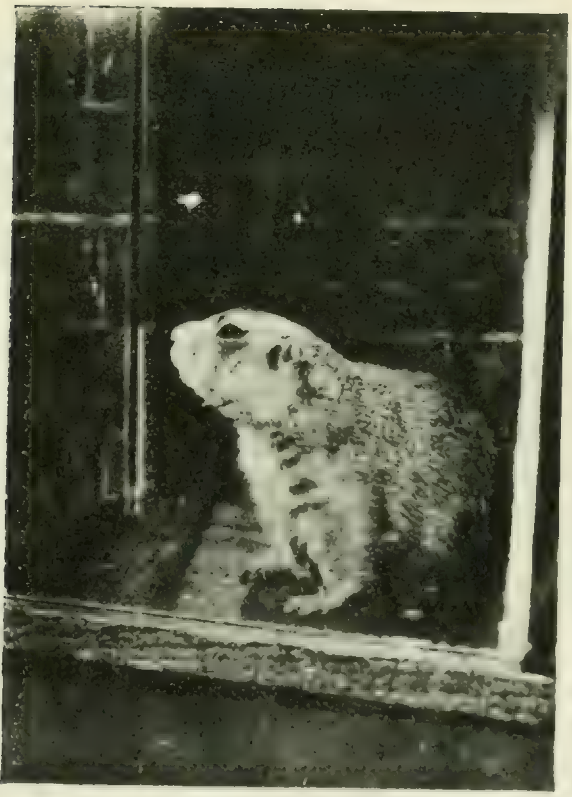

Fig. 2.

(McCollum and Parsons: Antiscorbutic requirement of prairie dog.)

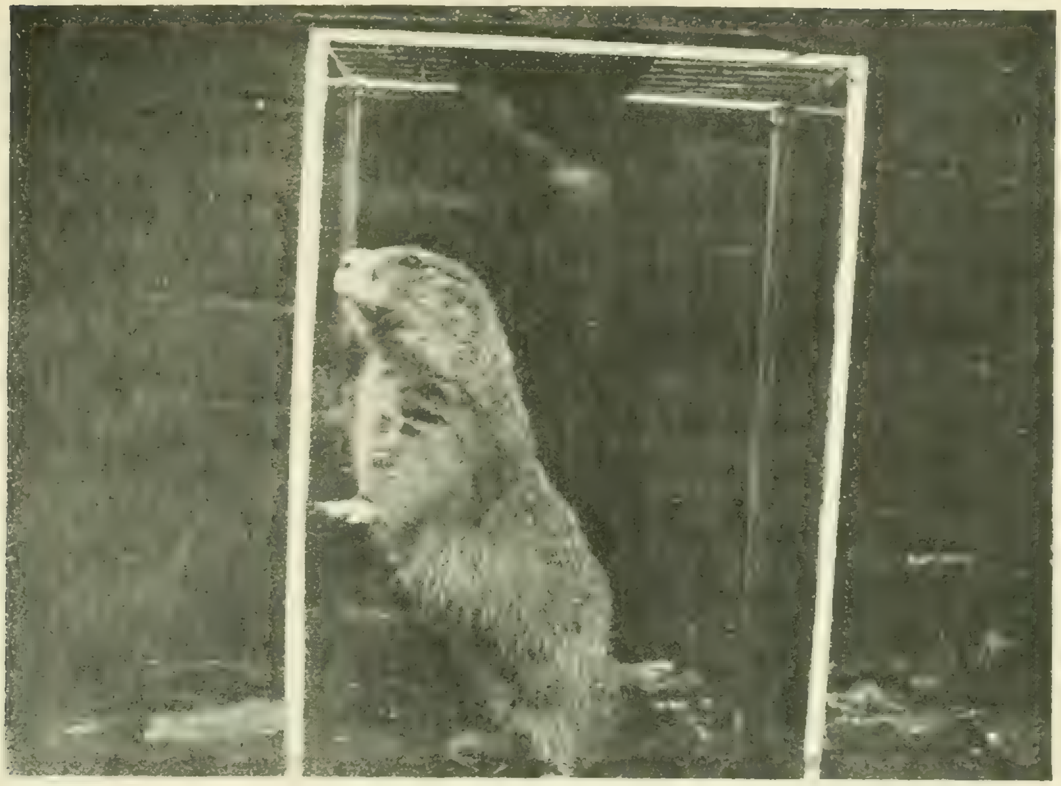

Fig. 3.

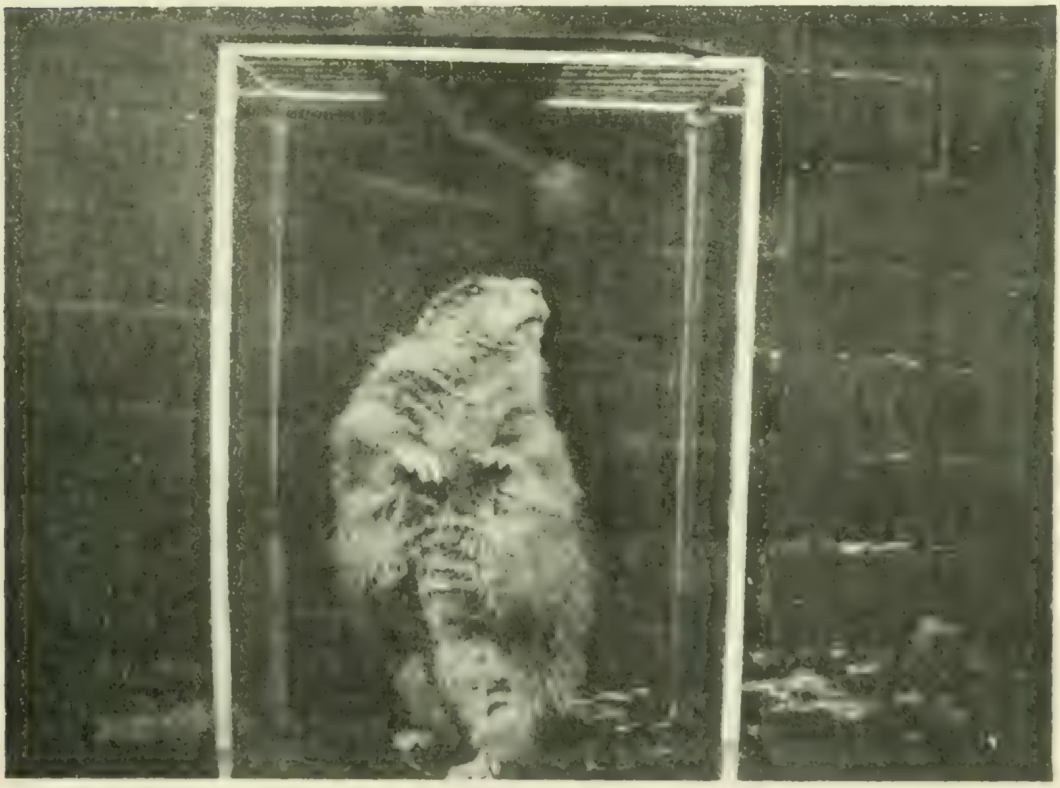

Fig. 4.

(McCollum and Parsons: Antiscorbutic requirement of prairie dog.)

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SUPPLEMENTARY PROTEIN VALUES IN FOODS.

I. THE NUTRITIVE PROPERTIES OF ANIMAL TISSUES.

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In 1915 McCollum and Davis[1] described a systematic procedure for evaluating each of the several essential factors in foodstuffs. This procedure involves the feeding of the food under consideration as the sole source of nutriment to one group of animals, and to another the same food supplemented with single and multiple additions of purified foodstuffs in every possible combination. Such additions include protein, inorganic salts, a source of fat-soluble A, water-soluble B, and as was later pointed out by Chick and her coworkers,[2] Cohen and Mendel,[3] and others, water-soluble C. The latter factor is not essential in the diet of the rat. This procedure constitutes a biological method for the analysis of a foodstuff, and has been adopted by several students of nutrition. It has yielded results which have profoundly changed our basis of judgment as to the quality of a diet.

Studies from several laboratories have established the general landmarks which enable us to appreciate the lines of procedure which must be followed if satisfactory diets are to be made up by combining the various types of animal and vegetable foods. In order to make such combinations of foodstuffs it is necessary that we should understand in detail the special qualities of each of the important natural foods. Such an understanding can be secured only through carefully planned experiments on animals in which each food is studied as the sole source of nutriment, then studied in combination with each of the other foods with which it may be used in practice. It is necessary to proceed from the simple to the complex mixtures in these studies. Ultimately it is hoped that diets can be planned which will promote the optimum of physiological well being, and therefore lead to the optimum in physical development, length of life, and the preservation of youthful characteristics.

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[1] McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, xxiii, 181.

[2] Chick, H., and Hume, E. M., *Tr. Soc. Trop. Med. and Hyg.*, 1917, x, 141. Chick, H., Hume, E. M., and Skelton, R. F., *Biochem. J.*, 1918, xii, 131.

[3] Cohen, B., and Mendel, L. B., *J. Biol. Chem.*, 1918, xxxv, 425.

In publishing this series of papers dealing with the studies of the dietary properties of several types of food mixtures, several new observations will be pointed out. The interpretation of the results is based upon more careful and thorough observations than have hitherto been described in any similar studies. They include not only the rate and extent of growth, the fertility, and success in rearing of young, but also the period of life up to and including the onset of old age with its characteristic changes.

Since animal tissues have a very prominent place in the diet of man in most parts of the world, it is of great moment to understand the value of these with respect to each of the essential dietary factors. Our knowledge of the nutritive qualities of animal tissues is still very incomplete. Watson and Hunter[4] showed that rats fed exclusively on muscle meats suffered severe malnutrition. Liver has, however, found great favor as a food for young fish in hatcheries. It has been shown[5] that lard does not contain appreciable amounts of fat-soluble A, whereas fats extracted from a glandular organ (pig kidney or cod testicle) are a good source of it.[6] Liver and kidney have been shown[7] to be a good source of both fat-soluble A and water-soluble B, whereas muscle tissue is very poor in both.

Heart, a variety of muscle, was found on the other hand to contain sufficient of both fat-soluble A and water-soluble B to support growth for a time at least in young rats.[7]

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[4] Watson, C., and Hunter, A., *J. Physiol.*, 1906, xxxiv, 111.

[5] McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1913, xv, 167. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1913–14, xvi, 423.

[6] McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, xx, 641.

[7] McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, xxi, 179. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxxii, 309; 1918, xxxiv, 17.

McCollum[8] pointed out that the mineral content of animal tissues such as muscle and glandular organs resembles that of seeds of plants sufficiently to indicate that it would not prove satisfactory as a source of inorganic elements in animal nutrition.

No data exist showing the comparative values of the proteins of the several kinds of animal tissues. Osborne and Mendel[7] have shown that growth takes place in young rats restricted to 18 per cent of protein derived solely from liver, kidney, muscle, or brain. This is a liberal amount, and is far above the plane of intake necessary for the support of normal growth in the rat when the quality of the protein is good.

There is also reason to inquire into the possibility of the presence of toxic substances in the glandular organs. The presence of such powerful physiological stimulants as thyroxin and adrenalin in the thyroid and suprarenal glands, respectively, makes them unfit for human or animal food. We have been informed that the Eskimos do not eat the liver of the reindeer. This fact may perhaps be accounted for by the absence of a gall bladder in this species, and consequent high content of bile, which would give it a bitter flavor. The Eskimos are said not to eat the liver of the polar bear, to avoid that of a certain species of seal, and to believe the liver of the dog poisonous. The liver is concerned with so many types of transformations of organic substances which have their origin in metabolism that it seemed possible that certain of these may be present in sufficient amounts to be detrimental to one who eats freely of it. Similar consideration might lead one to inquire whether the kidney of an animal may contain sufficient amounts of certain metabolic products as to render it undesirable as a food.

The glandular organs are rich in cell nuclei and consequently yield considerable amounts of purines when metabolized. These ultimately are converted in great measure into uric acid. There are conditions of perverted metabolism in man in which the excretion of uric acid and urates is interfered with and doubtless under such circumstances liver or kidney should not be eaten. There would seem little reason, however, why these organs should not be eaten by healthy persons as adjuvants to the diet. They possess dietary properties as distinct from those of muscle tissue as the leaves of plants do in contrast to the seeds.

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[8] McCollum, E. V., The newer knowledge of nutrition, New York, 1918.

In regions such as Labrador and Newfoundland where the diet of the habitants consists essentially of wheat flour, molasses, fish, meats, tea, and raisins, beri-beri and scurvy are common. A condition popularly called night-blindness is also of frequent occurrence among these people. On such a diet one would suspect the danger of developing xerophthalmia and apparently this is the case. The successful treatment of night-blindness has recently been reported by the administration of cod liver oil. This oil is a good source of fat-soluble A and is very effective in the cure of xerophthalmia.

Fresh liver is rich in fat soluble-A, water-soluble B, and water-soluble C, the protective dietary factors for ophthalmai, beri-beri, and scurvy, respectively. It should be easily possible to eradicate these dietary diseases in such regions as Labrador and Newfoundland by the use of fish livers as food. It is strange indeed that the natives of these regions have failed to discover the value of a by-product of their fishing industry which would serve in a great measure to correct the conspicuous faults in their diet.

For the purpose of comparing the biological values of the proteins of kidney, liver, and muscle, we have followed the procedure described by McCollum, Simmonds, and Parsons[9] of feeding the tissues singly as the sole sources of protein at planes so as to introduce 9 per cent of protein into the food mixture. They were supplemented with respect to all other factors so as to make a satisfactory diet, with the possible exception of the protein moiety. Lots 2475, 2476, and 2474, Chart 1, represent experiments of this type. Such experiments make it possible to compare the proteins of these animal tissues with those of a number of combinations of cereal and other seed proteins with milk which we have previously studied.[10] It has been shown that normal growth is secured when the diet contains 9 per cent of protein of high biological value. If much less than this content of protein is furnished by the food mixture, even when the protein is excellent, the growth falls distinctly below the curve of normal expectation.

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[9] McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1919, xxxvii, 155.

[10] McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, xx, 415.

When proteins of several types such as those of cereals, legume seeds, and milk were compared in this manner it was found that the proteins of milk were superior to those of most seeds of plants yet examined. The seed which furnishes proteins nearest to milk in value for conversion into body proteins during growth is wheat, which therefore stands first among the cereals in value. From the records of Chart 1, which show the effects of feeding kidney, liver, and muscle proteins respectively at 9 per cent of the food mixture, it is apparent that the proteins of these substances are scarcely superior to those of certain cereals, especially wheat. We base this conclusion on the growth of young rats restricted to one of these sources of protein at the critical 9 per cent level. Normal growth is not secured with wheat proteins fed below this plane of intake.

This result is very surprising indeed. It seems best explainable on the assumption that the patterns on which the proteins of the muscle are constructed differ very decidedly from that of the liver or kidney, and presumably from the glandular organs as a class. The nutritive needs of the body involve the replacement of tissues of both organ and muscle types. Apparently glandular organs or muscle as the sole source of nitrogen in the food fails to serve for efficient transformation into new body proteins during the symmetrical growth of the body tissues. They are complete but their transformation into body proteins cannot be very effectively accomplished (Chart 1).

We have shown in a previous communication that the cereal grain proteins do not in general make good each other's deficiencies or enhance to any great extent each other's biological values when two of them are combined.[9] Rye and flaxseed meal proteins form a notable exception. These, when combined in certain proportions, form a mixture which is distinctly superior to the proteins of either component alone. The supplementary value of one protein for another depends on the yields by each of those indispensable amino-acids which are present in each of the sources in smallest amounts. We shall show in one of the following papers that kidney, liver, and muscle proteins have much greater values as supplements to the cereal proteins than the cereal or legume proteins have, with few exceptions, among themselves. The data in the succeeding paper illustrate the importance of animal tissues in the food supply when the diet consists mainly of such vegetable products as do not yield a mixture of proteins having a high biological value. For the special purpose of enhancing the quality of the protein in the diet they have the highest value. It must be kept in mind, however, that their use does not correct the mineral deficiencies of a cereal, tuber, and legume seed diet, and that when muscle meats are used as food they have little effect in raising the content of fat-soluble A in the resulting mixtures. In no instance, therefore, will one of these types of animal tissue supplement a cereal, tuber, and fleshy root type of diet so as to make it highly satisfactory.

Our observations on the rats described in these experiments do not show any definite evidence of injury to the animals as the result of being fed excessively high protein diets. These diets were, however, essentially complete and fairly well proportioned as regards all factors other than protein. It is not justifiable to generalize from these results that such a high protein intake is safe for man. Our animals were not kept to determine the possible span of life or the time of appearance of the signs of senility, owing to the necessity of temporarily vacating the room in which the animals were kept. It is the custom for people in the United States to derive a high protein diet, when such is taken, in great measure from muscle meats, fish, poultry, eggs, and legume seeds. Menus containing such high protein foods will only in exceptional cases be completely supplemented by other constituents of the diet. The evil effects often attributed to excessive protein consumption may now with some confidence be attributed in many instances to faults in the composition of the diet in factors other than protein. Further studies are required to demonstrate the relative merits of diets of high and low protein contents when other factors are of comparable value in the two cases. We shall discuss this phase of nutrition on the basis of carefully planned experiments on animals in a later communication.

There are some very interesting and important instances of successful nutrition among people who have lived almost exclusively upon a diet very rich in protein and derived from foods of animal origin. We are indebted to Mr. Vilhjalmur Stefansson for the information that previous to about 1850 dental caries were very rare or absent from Iceland. During the last half of the 19th century cases have gradually become more and more common until today infected teeth are perhaps as common there as in most parts of the United States. No carious teeth were found among 96 skulls disinterred by Stefansson from a cemetery in Iceland dating from the 9th to the 13th centuries. These skulls are now in the Peabody museum at Harvard University and have been described by Hooton.[11] The diet of the Icelanders previous to about 1850 consisted essentially of milk, mutton, fish, and fowl, but in some parts of the island they ate the eggs of wild birds. The only vegetable food eaten regularly was carrageen moss, but potatoes and turnips were eaten to some extent. The teeth of the natives and their general health were excellent as long as this diet was taken. The deterioration of the teeth apparently began about the time when cereals and sugar were regularly imported into Iceland as sources of food.

The teeth of the primitive Eskimo were excellent. The younger generation in northern Alaska, whose diet is derived in a large measure from cereal grain products, canned foods, and muscle meats, similar to what would be purchased in a grocery store in the United States, has poorly calcified teeth which are often carious.

We have collected numerous observations on the effect of dietary faults on the quality of the skeleton in the rat. These data make it clear that most profound differences in the extent of calcification and density of the deposited calcium phosphate can be effected by such faults as are found in the cereal, tuber, and muscle meat type of diet.

The diet of the primitive Eskimo was very rich in protein but it was at least fairly satisfactory with respect to other factors. It consisted of muscle tissue and fat as the principal components, but all blood was carefully saved and eaten, and the glandular structures were regarded as dainties of especial delicacy. In addition, they regularly ate bone marrow and chewed the softer parts of bones, such as ribs and the epiphyses of the long bones. Such a selection of tissues suffices for the satisfactory nutrition of the rat and produces the fine physical development seen in the Carnivora such as the lion, tiger, jaguar, etc. The deterioration of the teeth of the Eskimo which occurred simultaneously with the modification of the diet due to contact with the white man is in harmony with what we have been led to expect as the result of experimental studies of the types of combinations of ordinary foods which enter into the diet of man and animals in different parts of the world. The cereal and muscle meat diet or its equivalent, the bread, meat, and potato type of diet, is in all probability the cause of the deterioration of the teeth of the present generation of "civilized" Eskimos as it is among the people of the United States and Europe.

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[11] Hooton, E. A., *Am. J. Physical Anthropol.*, 1918, I, 53.

CONCLUSIONS.

The kidney, liver, and muscle of the ox contain proteins which, when they serve as the sole source of nitrogen, and are fed singly as the sole source of protein, but completely supplemented with respect to all necessary factors other than protein, are shown to possess about the same biological value as those of the wheat kernel.

There is no distinct evidence of toxicity in either muscle, kidney, or liver tissue when fed at planes of intake sufficiently high to introduce from 35 to 70 per cent of protein into the diet.

The first limiting factor in the kidney, liver, and muscle tissue is a lack of calcium. It is also necessary to add sodium chloride in order to insure prolonged well being. Carnivorous man and animals secure their sodium chloride by eating blood, and calcium by eating bone. Liver and kidney contain an abundance of fat-soluble A and of water-soluble B, and when fresh and raw, of water-soluble C. Muscle tissue is very deficient in these factors but does not entirely lack any one of them. Kidney proteins appear to have higher biological value than those of the other animal tissues yet studied.

It has been our custom for years in preparing experimental diets to thoroughly grind the several components of the food and to make a uniform mixture from which the constituent parts cannot be picked out by the animals. In all cases iodine was given once a week in the form of potassium iodide-iodine in the drinking water, which was distilled. The liver, kidney, and steak, except when the contrary is stated, were steamed in a sterilizer until thoroughly cooked, subsequently dried, and ground. Practically all visible fat was removed from both organs and muscle. The curves presented in the charts are typical representatives of a group of four to six animals which composed each experimental group.

Chart 1.—The curves in this chart illustrate the growth of young rats fed diets containing 9 per cent of protein derived solely from beef kidney, liver, and muscle, respectively. The inorganic additions were of a character which completed, at least in a fairly satisfactory manner, the mineral content of these animal tissues. There can be no doubt that kidney proteins have a somewhat higher biological value than those of liver. Liver proteins, surprising as it may seem, have not been found in our experiments to be superior to those of muscle (steak).

In Lot 2475 there were two females. One of these died in parturition. The other at the age of 4 months had one litter of four young and successfully reared them. She died from unknown cause about 40 days after weaning her young. Two of her daughters grew up and produced one and three litters of young (a total of seventeen), respectively. Of these ten were successfully weaned.

Two granddaughters of the female described in the original experimental group were kept 6½ months on the family ration, and although they appeared to be in good condition, neither proved fertile. The rats fed the kidney ration did not exhibit early signs of aging. In this respect they were superior to the groups fed diets containing comparable amounts of liver and of muscle.

The records of these experimental groups fed 9 per cent of protein from kidney, liver, and muscle, respectively, all other factors being more or less satisfactorily adjusted, show them to be typical examples of nutritional instability. In Lot 2475 on the kidney diet, each succeeding generation was inferior to its parents. Lack of uniformity of vitality among individuals of the same group or family is observed with striking frequency in animals whose diets fall but little below the quality necessary to maintain the vigor of the species unimpaired throughout successive generations.

It might be suggested that the failure of the animals fed liver to develop more satisfactorily, was due to the presence of toxic substances in this organ, which performs the function of degrading numerous foreign and poisonous substances derived from metabolism and absorption from the alimentary tract and elsewhere. The records of Chart 2 show clearly that this is not the case.

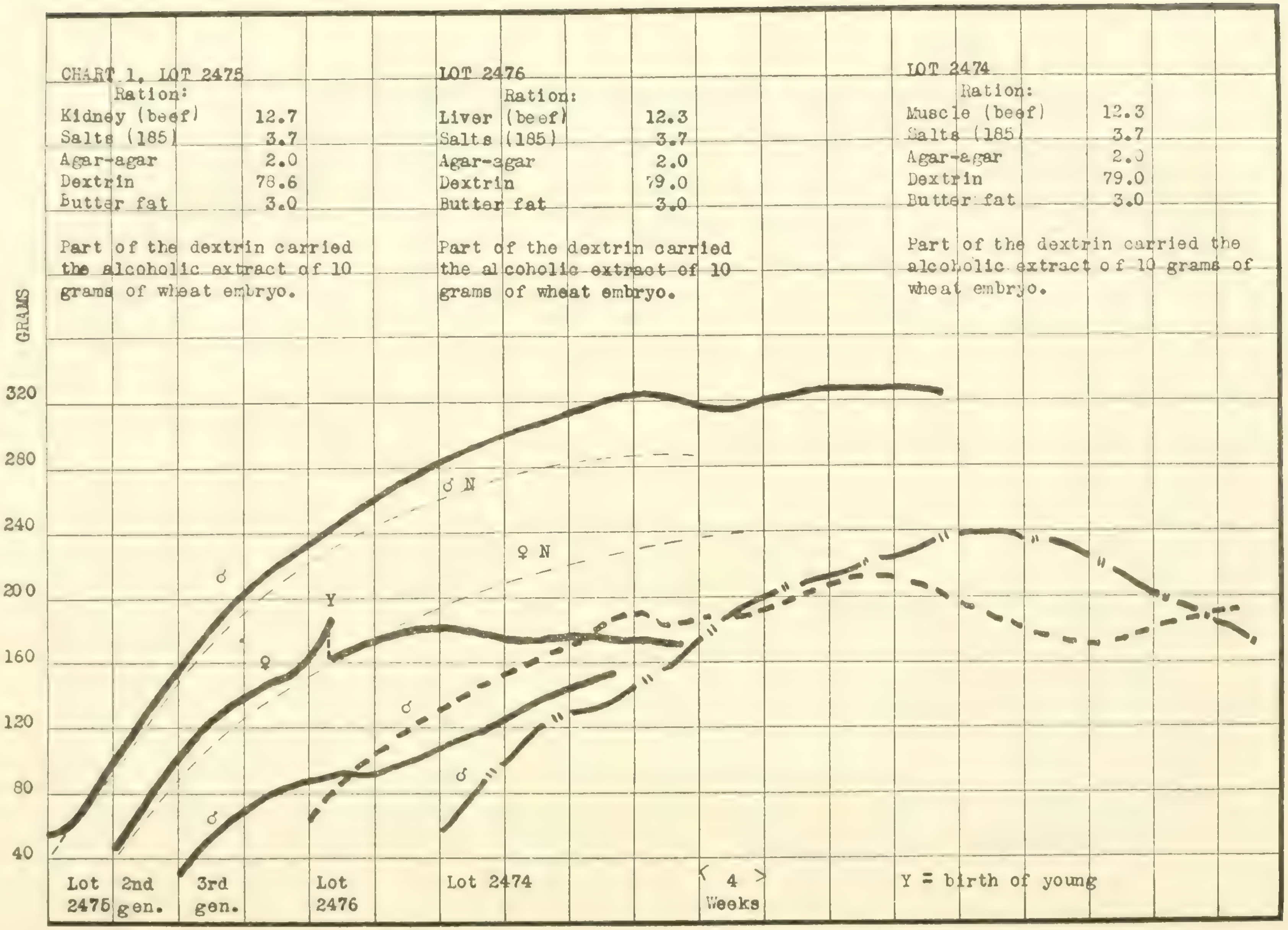


CHART 1. LOT 2475

Ration:	
Kidney (beef)	12.7
Salts (185)	3.7
Agar-agar	2.0
Dextrin	78.6
Butter fat	3.0

Part of the dextrin carried the alcoholic extract of 10 grams of wheat embryo.

LOT 2476

Ration:	
Liver (beef)	12.3
Salts (185)	3.7
Agar-agar	2.0
Dextrin	79.0
Butter fat	3.0

Part of the dextrin carried the alcoholic extract of 10 grams of wheat embryo.

LOT 2474

Ration:	
Muscle (beef)	12.3
Salts (185)	3.7
Agar-agar	2.0
Dextrin	79.0
Butter fat	3.0

Part of the dextrin carried the alcoholic extract of 10 grams of wheat embryo.

Y = birth of young

Neither of the two females fed the steak diet had any young, although they appeared to be well nourished and were kept under observation during more than 12 months.

Three females on the liver diet were somewhat undersized, aged early, and never had any young, although they were kept under observation for more than a year. In the case of both the liver and muscle diets this was apparently due to the proteins from these sources not being of sufficiently good quality to make 9 per cent of protein from these sources sufficient to promote well being at the optimum, since in these diets all other factors were corrected.

Composition of Salt Mixture 185.

	per cent
NaCl	0.173
$MgSO_4$ (anhydrous)	0.266
$NaH_2PO_4 + H_2O$	0.347
K_2HPO_4	0.954
$CaH_4(PO_4)_2H_2$	0.540
Fe citrate	0.118
Ca lactate	1.300

Chart 2.—The rats in these experiments were fed food mixtures which were satisfactorily constituted except for possible shortage of water-soluble B and fat-soluble A, which in each case were derived entirely from 25 per cent of kidney, liver, or muscle, respectively. The growth curves and fertility of the animals on the kidney and liver diets show that these amounts were satisfactory as sources of fat-soluble A and water-soluble B. In these diets the protein of the animal tissues was supplemented with 9 per cent of casein. Chart 4 shows, however, that 20 per cent of either kidney or liver suffices as the sole source of protein for normal growth, reproduction, and rearing of young, for the fourth generation on the kidney diet appeared to have normal vitality. The rats of the fourth generation on the liver diet were inferior, although they were successfully weaned.

Two females on the kidney diet, Lot 2163, had collectively four litters (fourteen young), all of which were reared. Two of the daughters were maintained during 10 months on the diet on which their mothers had lived. One remained sterile, the other had a single litter(five young) at about 4 months of age and never became pregnant afterwards. The young were in good condition when weaned.

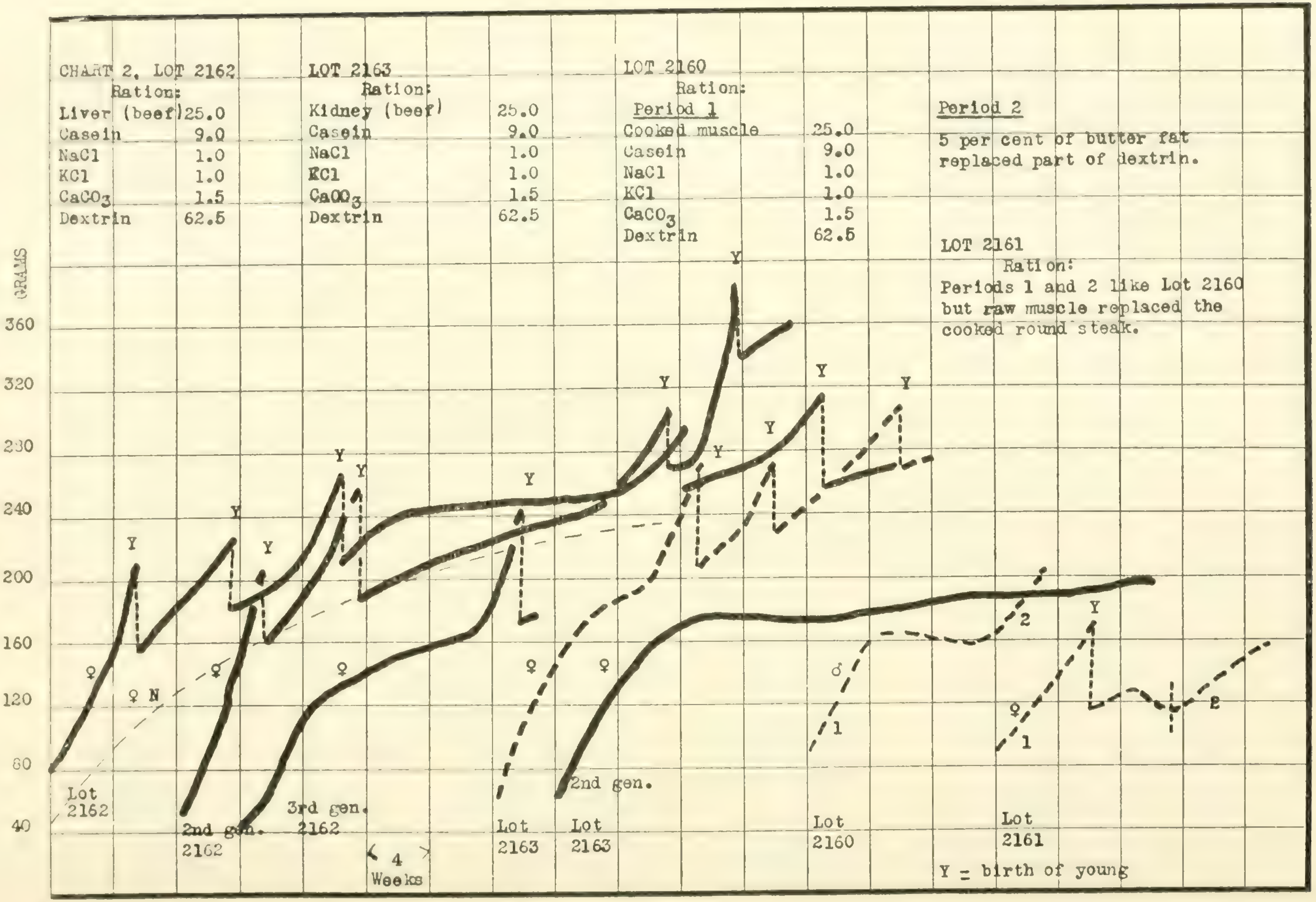


CHART 2. LOT 2162
Ration:
Liver (beef) 25.0
Casein 9.0
NaCl 1.0
KCl 1.0
CaCO3 1.5
Dextrin 62.5
LOT 2163
Ration:
Kidney (beef) 25.0
Casein 9.0
NaCl 1.0
KCl 1.0
CaCO3 1.5
Dextrin 62.5
LOT 2160
Ration:
Period 1
Cooked muscle 25.0
Casein 9.0
NaCl 1.0
KCl 1.0
CaCO3 1.5
Dextrin 62.5
Period 2
5 per cent of butter fat replaced part of dextrin.
LOT 2161
Ration:
Periods 1 and 2 like Lot 2160 but raw muscle replaced the cooked round steak.
GRAMS
360
320
230
240
200
160
120
80
40
Y
♀
♀ N
♂
1
2
Lot 2162
2nd gen. 2162
3rd gen. 2162
4 Weeks
Lot 2163
Lot 2163
2nd gen.
Lot 2160
Lot 2161
Y = birth of young

There were three females raised on the liver diet (Lot 2162). They had collectively fifty-six young (nine litters), of which thirty-three were weaned. The young which died became lethargic just before weaning time and died in this condition. We have at present no explanation for this peculiar behavior.

Lots 2160 and 2161 were fed diets comparable to Lots 2162 and 2163, except that the factors, fat-soluble A and water-soluble B, were entirely derived from 25 per cent of muscle tissue (cooked and raw beefsteak, respectively). In both cases there was failure of growth after about 4 weeks. After a period of suspended growth during which one female in Lot 2161 had a litter of four young which were eaten by the mother shortly after birth, 5 per cent of butter fat was added to the diet of each group. In both cases there was a marked response to growth following this addition. This demonstrates that 25 per cent of round steak does not contain sufficient fat-soluble A to meet the needs of the young rat. Lot 2142, Chart 3, shows clearly that 20 per cent of steak does not furnish sufficient water-soluble B for the normal nutrition of young rats during growth.

The records in this chart show clearly the remarkable difference between the glandular organs as compared with muscle tissue in respect to their content of both the factors, fat-soluble A and water-soluble B. This supports the view which we have repeatedly stated, that the dietary properties of a substance can be fairly accurately predicted from a knowledge of their biological function.

Chart 3.—The three groups of animals whose curves are shown in this chart were fed diets comparable in all respects, except that the sole source of water-soluble B and protein was 20 per cent of the dry matter in the diet in the form of kidney, liver, and muscle, respectively. A comparison of these curves with those of Chart 1 shows that the protein furnished by this proportion of liver, kidney, and steak, respectively, suffices to support normal growth when other factors in the diet are properly adjusted.

The animals fed the kidney ration, Lot 2144, were markedly superior to the other two groups. Representatives of four successive generations were grown upon this diet as their sole source of water-soluble B, and with no evidence of deterioration. In the original group there were three females. These produced collectively ten litters (forty young) but only six individuals were

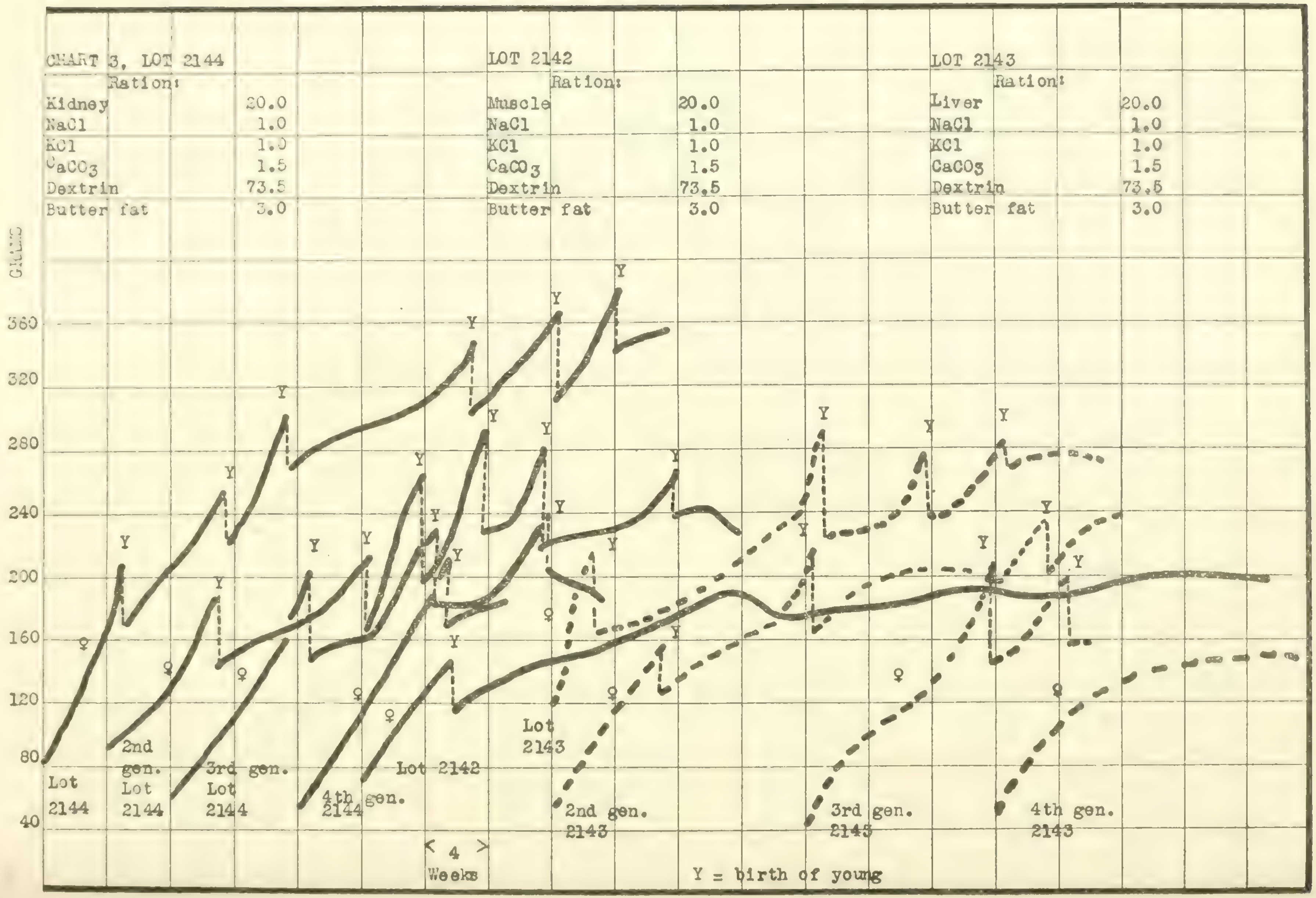


CHART 3, LOT 2144
Ration:
Kidney 20.0
NaCl 1.0
KCl 1.0
CaCO3 1.5
Dextrin 73.5
Butter fat 3.0
LOT 2142
Ration:
Muscle 20.0
NaCl 1.0
KCl 1.0
CaCO3 1.5
Dextrin 73.5
Butter fat 3.0
LOT 2143
Ration:
Liver 20.0
NaCl 1.0
KCl 1.0
CaCO3 1.5
Dextrin 73.5
Butter fat 3.0
GRAMS
360
320
280
240
200
160
120
80
40
Y
♀
Lot 2144
2nd gen. Lot 2144
3rd gen. Lot 2144
4th gen. 2144
Lot 2142
Lot 2143
2nd gen. 2143
3rd gen. 2143
4th gen. 2143
< 4 > Weeks
Y = birth of young

successfully weaned. The mothers destroyed their young soon after birth.

Two daughters of the females just described were kept on the family diet. They had together six litters (forty young) and weaned only four individuals altogether. Here again the mortality was the result of cannibalistic tendencies in the mothers. But one granddaughter of the original group was kept on the family diet. She grew normally and had six young (two litters) and successfully weaned all of them. One great granddaughter was brought up on the diet. She had three young (one litter) and weaned them successfully. With optimum nutrition these litters would have been two or three times as large as those produced.

It might be suggested that perhaps the content of water-soluble B in this diet was below the optimum, and that for this reason the nutrition of the nervous systems of the rats restricted to this diet was faulty, and that the tendency to destroy the young was an expression of an abnormal psychology analogous to the psychosis observed in beri-beri or pellagra. The fact that the third and fourth generation females in this family were less cannibalistic than their mothers and grandmothers militates somewhat against this view.

Lot 2143 derived all its antineuritic factor and protein from 20 per cent of liver in the diet. This was equivalent to 14.4 per cent of protein. The growth curves were normal. In the first group fed this diet there were two females. These had forty-six young (eight litters) and of these but twenty were weaned. Two second generation females had eighteen young (four litters) and weaned ten of them. Two third generation females had thirteen young (two litters) of which they weaned six. One fourth generation female was kept 5½ months, when she died, never having had any young. None of these animals in any generation was kept to an advanced age. The young of the rats fed the liver diet were somewhat undersized but appeared to be vigorous.

Lot 2142 was fed a diet comparable to the others described in this chart, but with all protein and water-soluble B derived from 20 per cent of muscle (round steak). These animals were markedly inferior both as regards growth and fertility to those fed kidney or liver at the same plane of intake. Two females were kept to the age of 15 months, an age which usually marks the end of fertility in the rat. One had a single litter of eight young but they died when about 2 weeks old. The other remained sterile. The rats in this group were still in good nutritive condition after a year on the diet. This chart, like the other records in this paper, shows clearly the superiority of glandular organs over muscle tissue as sources of water-soluble B.

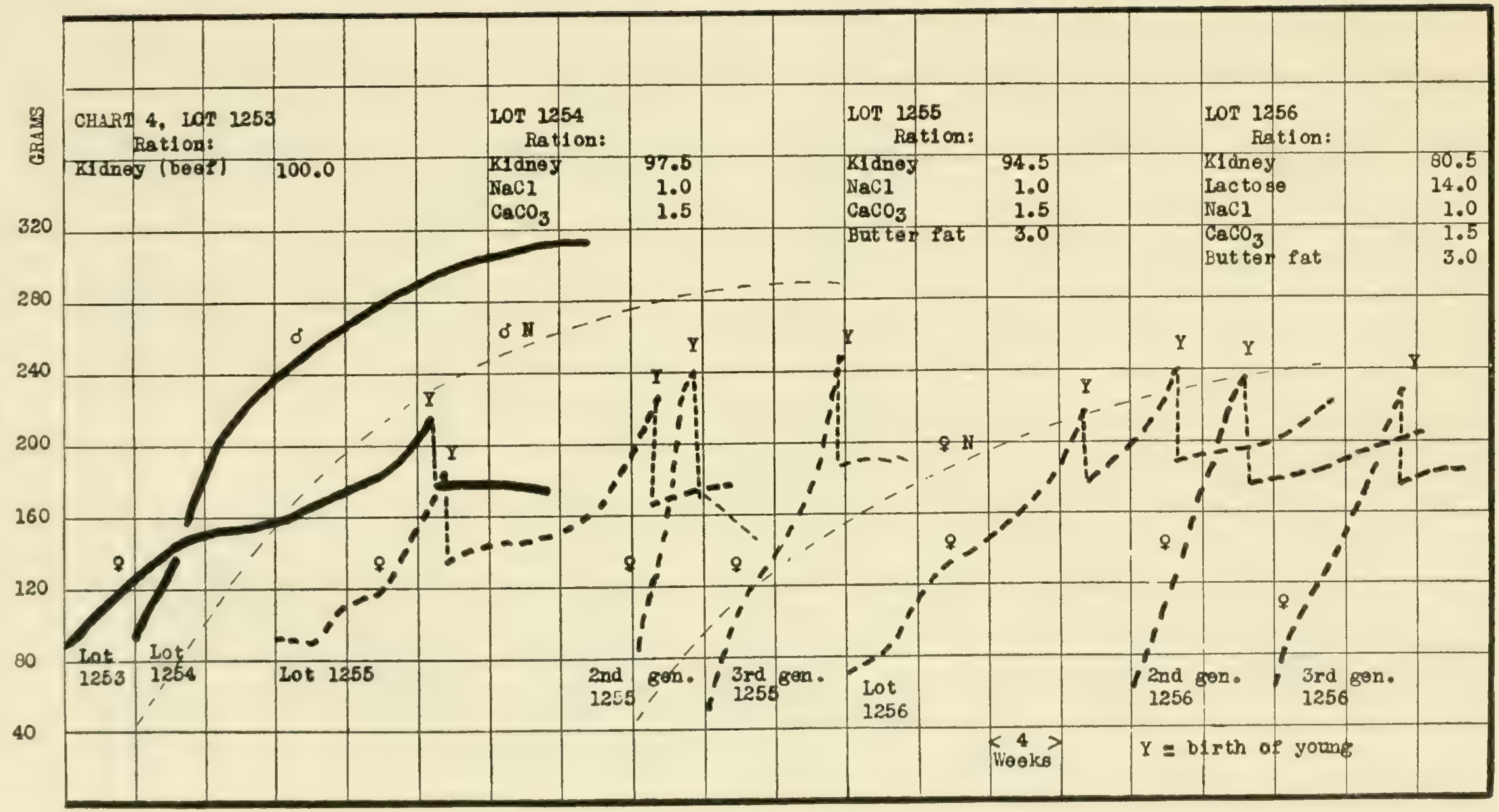
Chart 4.—These records show the behavior of young rats fed cooked dry beef kidney as the sole food, Lot 1253. Lot 1254 shows the growth curves of rats fed 97.5 per cent of kidney supplemented with sodium chloride and calcium carbonate. Lot 1255 was fed kidney, 94.5 per cent, supplemented with sodium chloride, calcium carbonate, and butter fat. Lot 1256 was fed a diet like that of Lot 1255 except that 14 per cent of the kidney was replaced by lactose. This last ration was designed to show whether lactose would tend to modify the bacterial flora of the alimentary tract. No beneficial effects of the modification were apparent.

The rats restricted to cooked dried kidney as their sole food grew in a fairly normal manner. Two females had collectively fourteen young (a litter each), five of which were weaned. One of these young developed abnormal ribs suggestive of rickets. None of these young grew up.

It is very remarkable that young rats confined to a diet of kidney could develop so successfully and reproduce and rear young. This tissue is very poor in calcium, and yields a great excess of acid on being metabolized. The protein content of this diet was not far from 71 per cent, yet because all essential food factors except calcium were so abundant, the animals were able to tolerate this deficiency and the abnormal protein content remarkably well.

Lot 1254 contained two females, one of which became pregnant and died in parturition. The other had a tumor which became so large that she was chloroformed.

There was but one female in Lot 1255. She had sixteen young (two litters) of which nine were weaned. Two second generation females had collectively seventeen young (a litter each) of which ten were weaned. Two third generation females had each a litter of young (seventeen) of which twelve individuals were weaned. The young appeared to be well developed, but were always greasy from their food. They drank much water. The cage had a strong odor. The protein content of the ration of Lot 1254 was 69 per


GRAMS
320
280
240
200
160
120
80
40
CHART 4, LOT 1253
Ration:
Kidney (beef) 100.0
LOT 1254
Ration:
Kidney 97.5
NaCl 1.0
CaCO3 1.5
LOT 1255
Ration:
Kidney 94.5
NaCl 1.0
CaCO3 1.5
Butter fat 3.0
LOT 1256
Ration:
Kidney 80.5
Lactose 14.0
NaCl 1.0
CaCO3 1.5
Butter fat 3.0
♂
♂ N
♀
Y
♀ N
Lot 1253
Lot 1254
Lot 1255
2nd gen. 1255
3rd gen. 1255
Lot 1256
2nd gen. 1256
3rd gen. 1256
< 4 > Weeks
Y = birth of young

cent; that of Lot 1255, 67 per cent; of Lot 1256, 57 per cent. The results show that the rat is capable of growing and remaining in a state of health on a diet comparable to that of the strictly carnivorous animals. At least among the carnivorous animals of the Arctic regions the proportion of protein in the diet is not necessarily excessive at all times. Mr. Stefansson has informed us that it is not unusual for travelers to come upon a seal that has been killed and skinned, the subcutaneous layer of fat eaten, and the remainder of the carcass left practically untouched. The polar bear evidently prefers fat to protein as a source of energy.

Lot 1256 contained two females. They had collectively twenty-two young (two litters each) of which fifteen were weaned; two second generation females had together nineteen young, and weaned fourteen of them. One third generation female had one litter of six young and weaned them all.

Chart 5.—The diet of the four groups of rats whose curves are shown in this chart was in all instances comparable to those in Chart 4, the only difference being the substitution of liver for kidney in the diets of Lots 1277, 1278, 1279, and 1280.

Lot 1277, which was confined to liver as its sole food, grew very little and died from 4 to 6 months after being confined to the diet.

Lot 1278 was fed liver supplemented with sodium chloride and calcium carbonate. On this diet growth was approximately normal. The group contained three females. One of these had twelve young (two litters) and weaned eleven of them. The other two remained sterile. One second generation female remained sterile.

Lot 1279 was fed liver supplemented with sodium chloride, calcium carbonate, and butter fat. Lot 2162, Chart 2, shows that even 25 per cent of liver as the sole source of fat-soluble A furnishes a sufficient amount of this factor.

There were three females in this group. They had thirty-four young (six litters) and weaned thirty-one of them. One second generation female grew up on the diet, and had six young in one litter. She weaned four of these. One third generation female in this family had a litter of six and weaned them all. The reproduction records of this group were distinctly better than those of Lot 1278, whose diet was identical except for the 3 per cent of but-

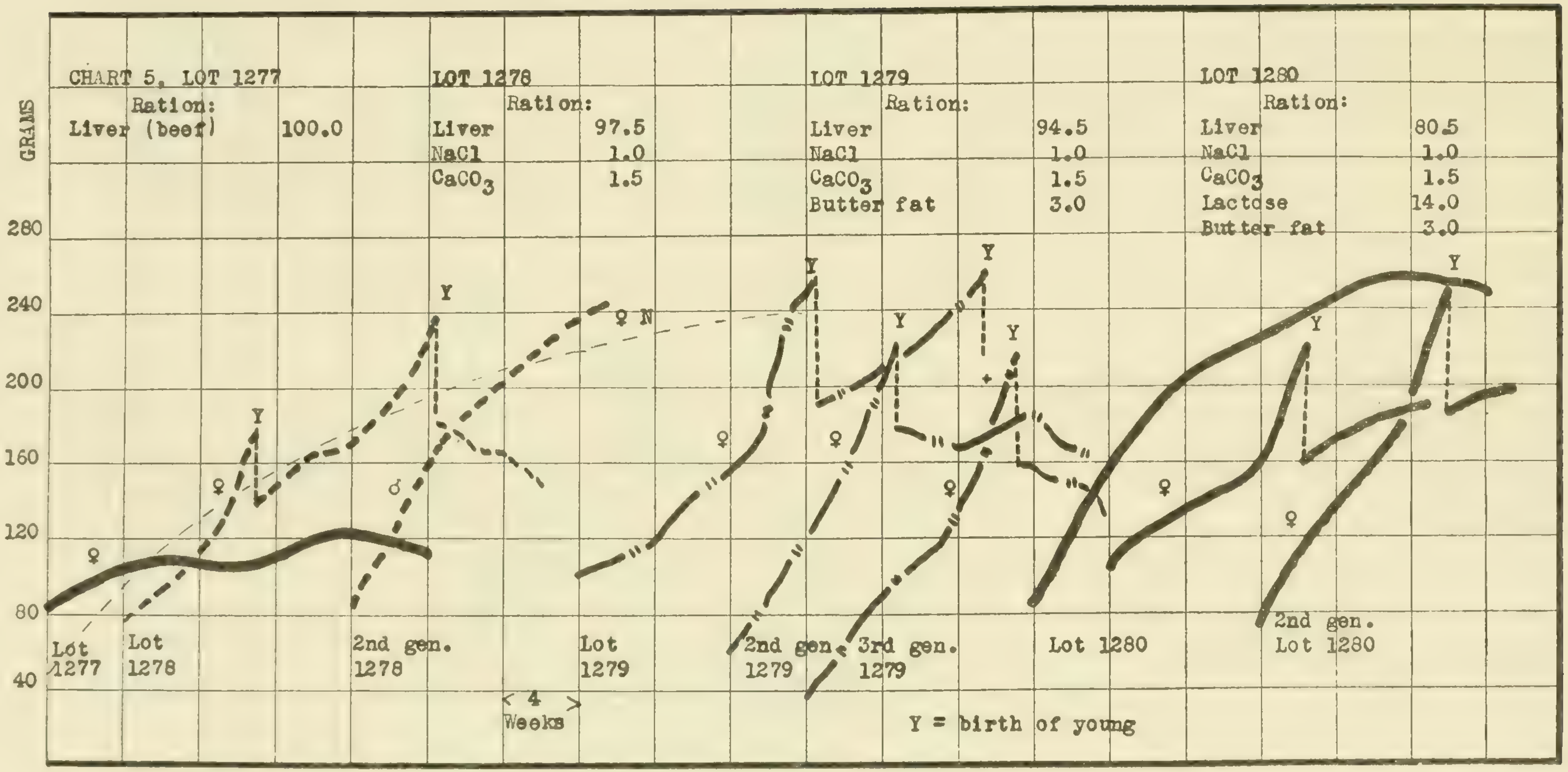


CHART 5, LOT 1277
Ration:
Liver (beef) 100.0
LOT 1278
Ration:
Liver 97.5
NaCl 1.0
CaCO3 1.5
LOT 1279
Ration:
Liver 94.5
NaCl 1.0
CaCO3 1.5
Butter fat 3.0
LOT 1280
Ration:
Liver 80.5
NaCl 1.0
CaCO3 1.5
Lactose 14.0
Butter fat 3.0
GRAMS
280
240
200
160
120
80
40
♀
♀
Y
♂
Y
♀ N
♀
Y
♀
Y
Y
♀
Y
♀
Y
♀
Y
Lot 1277
Lot 1278
2nd gen. 1278
Lot 1279
2nd gen. 1279
3rd gen. 1279
Lot 1280
2nd gen. Lot 1280
< 4 > Weeks
Y = birth of young

ter fat. We cannot explain the reason for the superiority of Lot 1279.

Lot 1280 was like Lot 1279 except that 14 per cent of lactose replaced a like amount of liver. This did not exert any noticeably favorable effect on the well being of the animals. Two females each had a litter (collectively fourteen young) and weaned thirteen individuals. One second generation female had one litter of eight young and weaned seven of these. The young in Lots 1279 and 1280 appeared to be strong and in good condition but showed in all cases a peculiar condition which we have observed occasionally in abnormal animals; *viz.*, a wet and stained area around the urethral orifice. This not infrequently occurs in poorly nourished animals.

Chart 6.—The animals whose records are shown in this chart were fed a diet made adequate in all respects as far as could be judged, and with the protein derived solely from 50 per cent of dry beef liver. The protein content of the diet was about 35 per cent.

Lot 1281 contained three females. These had collectively thirty-five young (five litters) and weaned twenty-two. Two second generation females had one litter each, collectively nineteen young, of which number they weaned seventeen. These young appeared to be very well nourished.

Lot 1282 contained two females. These collectively had five litters (thirty-four young). They weaned thirty-one of these young.

Two second generation females were restricted to this diet. One had a litter of six, which she successfully weaned. The other female remained sterile. The young were apparently well nourished but badly urine-stained. There was no evidence that the inclusion of lactose benefited the animals.

Chart 7.—The animals whose records are shown in this chart were fed either muscle tissue or blood, with and without certain purified food additions. Lot 1232 was restricted to a diet of cooked, dried, beef muscle. They were able to grow very slowly, but remained very much undersized, and died early. None of this group had any young. Two of the rats showed, toward the end of their lives, distinct signs of xerophthalmia, due to the lack of fat-soluble A in muscle tissue.

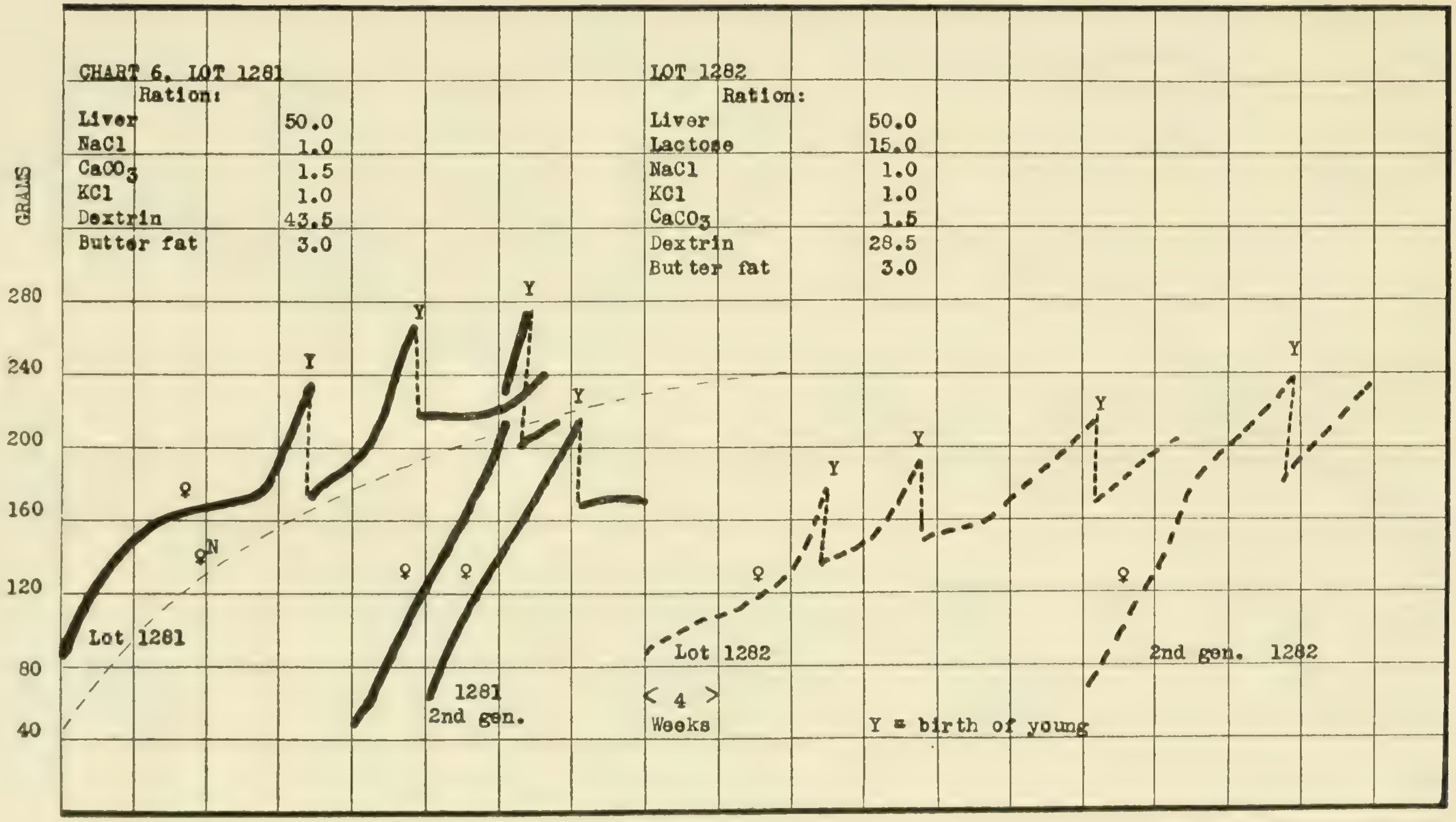


CHART 6. LOT 1281
Ration:
Liver 50.0
NaCl 1.0
CaCO3 1.5
KCl 1.0
Dextrin 43.5
Butter fat 3.0
LOT 1282
Ration:
Liver 50.0
Lactose 15.0
NaCl 1.0
KCl 1.0
CaCO3 1.5
Dextrin 28.5
Butter fat 3.0
GRAMS
280
240
200
160
120
80
40
Y
Y
Y
Y
Y
Y
Y
Y
Y
♀
♀N
♀
♀
♀
♀
Lot 1281
1281
2nd gen.
Lot 1282
2nd gen. 1282
< 4 >
Weeks
Y = birth of young

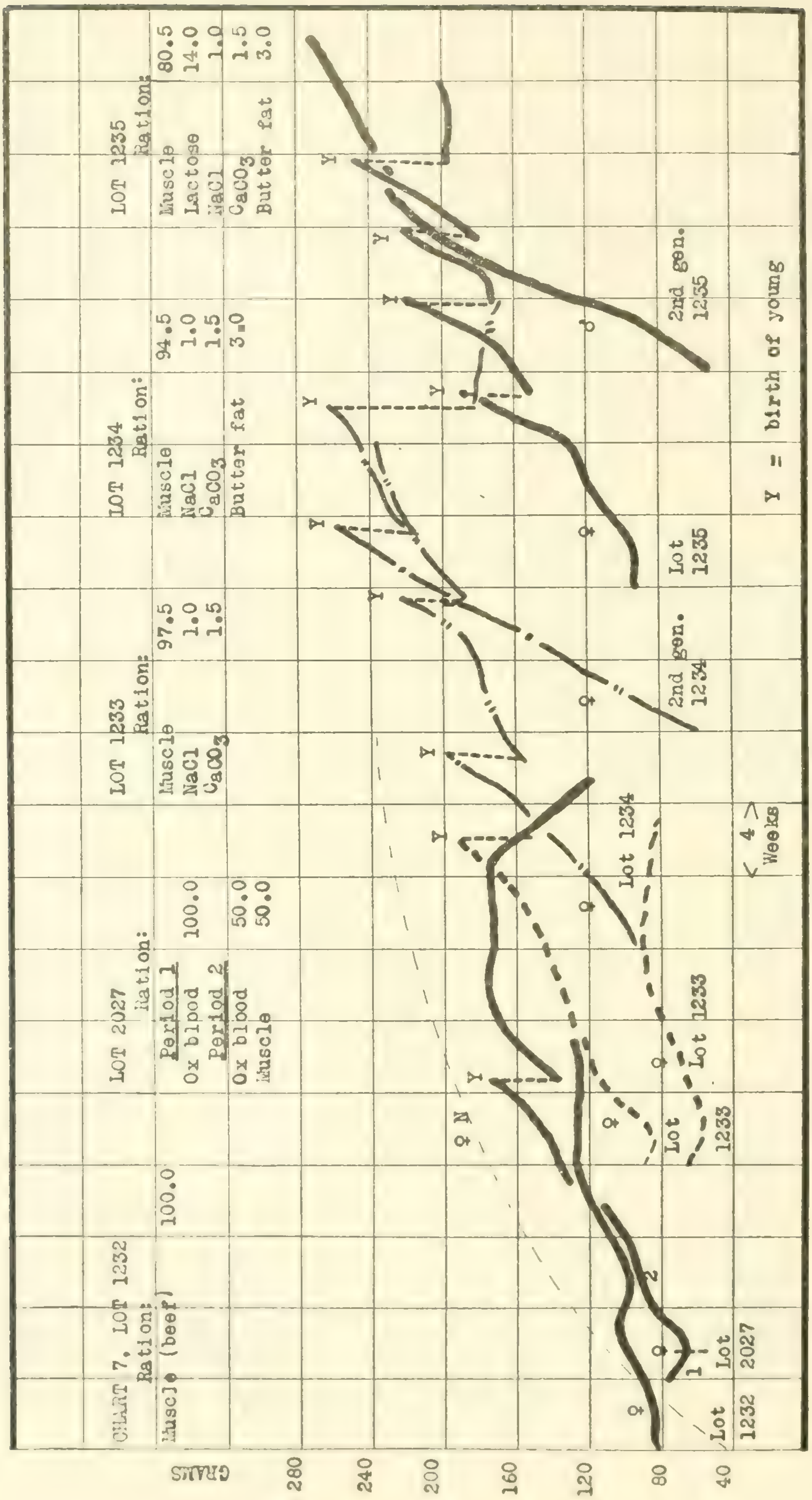


CHART 7. LOT 1232
Ration:
Muscle (beef) 100.0
LOT 2027
Ration:
Period 1
Ox blood 100.0
Period 2
Ox blood 50.0
Muscle 50.0
LOT 1233
Ration:
Muscle 97.5
NaCl 1.0
CaCO3 1.5
LOT 1234
Ration:
Muscle 94.5
NaCl 1.0
CaCO3 1.5
Butter fat 3.0
LOT 1235
Ration:
Muscle 80.5
Lactose 14.0
NaCl 1.0
CaCO3 1.5
Butter fat 3.0
GRAMS
280
240
200
160
120
80
40
Lot 1232
Lot 2027
1
2
Lot 1233
Lot 1233
Lot 1234
2nd gen. 1234
Lot 1235
2nd gen. 1235
♀
♀ N
Y
< 4 > Weeks
Y = birth of young

Lot 2027 was fed dried ox blood as their sole food in Period 1. They declined rapidly on this and after 2 weeks were changed to a diet of muscle meat 50 per cent and dried blood 50 per cent. On this diet they were able to grow at a rate approximately half the normal. A female in this group had a single litter of four young, which she destroyed within a few days. All these animals showed early signs of old age.

Lot 1233 was fed cooked dried muscle (beefsteak) supplemented with sodium chloride and calcium carbonate. They grew but little better on this diet than on muscle alone, but one female had a litter of three young, which she destroyed soon after birth. Two other females remained sterile on this diet. That fat-soluble A was the limiting factor in this diet is shown by the records of Lots 1234 and 1235.

Lot 1234 was fed muscle, sodium chloride, calcium carbonate, and butter fat. They not only grew in an approximately normal manner, but were fairly fertile and had moderate success in the rearing of their young. Two females had together twenty young (three litters) of which sixteen were weaned. One second generation female was kept on the diet. She had one litter of five young and weaned them all. The young were somewhat inferior. Further studies are necessary to determine what modifications of this diet are necessary to secure greater fertility and higher vitality in the young.

Lot 1235 was fed a diet of beefsteak supplemented with sodium chloride, calcium carbonate, butter fat, and 14 per cent of lactose. They were not superior in vigor, fertility, or success in rearing young to Lot 1234 which had more beefsteak in place of the lactose. Two females had thirty-two young collectively (five litters) of which twelve were weaned. Since these were all males no further reproduction records were secured.

Chart 8.—The animals whose records are shown in Chart 8 derived their protein and water-soluble B from 50 per cent of cooked, dried, muscle tissue (beefsteak). The protein content of the diet of Lot 1236 was about 35 per cent. The growth curves approximated the average and the animals appeared to be in a satisfactory state of nutrition.

There were two females in Lot 1236. Together they had thirty-four young (five litters) and weaned twenty-four of them. One

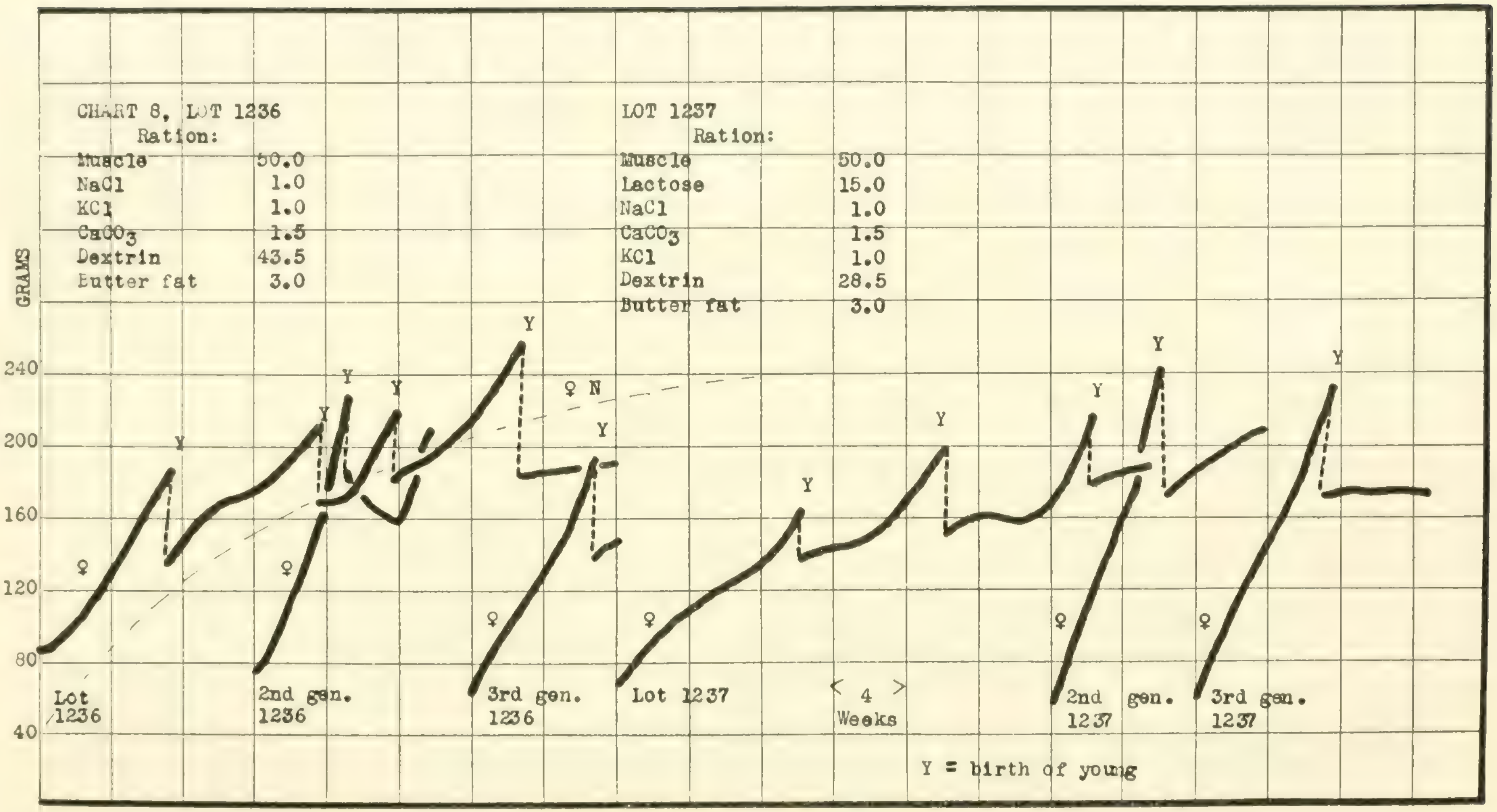


CHART 8, LOT 1236

Ration:

Muscle	50.0
NaCl	1.0
KCl	1.0
$CaCO_3$	1.5
Dextrin	43.5
Butter fat	3.0

LOT 1237

Ration:

Muscle	50.0
Lactose	15.0
NaCl	1.0
$CaCO_3$	1.5
KCl	1.0
Dextrin	28.5
Butter fat	3.0

daughter of one of these mothers was kept on the diet. She had one litter of eight young and weaned them all. Two of her daughters grew up on the diet and each had a litter, together thirteen young, and weaned but three of them. The young in most cases appeared normal. They were not urine-stained as many were on the liver diets.

Lot 1237 differed from Lot 1236 in that 15 per cent of lactose replaced an equivalent amount of dextrin. There was no evidence that this carbohydrate exerted any beneficial effect over dextrin.

Two females in this group had together thirty young (six litters) and successfully weaned them all. Two of the second generation females had each a litter (collectively fifteen young), of which eleven young were weaned. One third generation female had a litter of seven young and weaned them all.

Chart 9.—These records show the histories of three groups of young rats fed diets in which all protein and antineuritic factors were derived from raw muscle tissue (round steak). Lot 2056 was restricted in the first period to raw round steak as the sole source of nutriment. They grew no better on this than on cooked steak (Chart 7, Lot 1232). In a second period sodium chloride and calcium carbonate were added and these caused a slight response with growth for a time. The diet was too poor in fat-soluble A to admit of much growth. This is seen in the record of Lot 2058.

Lot 2058 was fed a diet containing 50 per cent of raw, dried muscle (beefsteak) supplemented with sodium, potassium, calcium, chlorine, and fat-soluble A in butter fat. The diet contained 43.5 per cent of dextrin. On this ration growth was normal and the animals were apparently in good nutritive condition. Three females each had a litter of young (collectively twenty-two) of which twenty were reared. Two second generation females each had a litter (fourteen young) and reared them all.

There was a period between the ages of 18 and 28 days when the young of the second generation appeared lethargic. They recovered later and appeared to be as alert as rats on the cooked steak. This was probably due to the effects of eating so much raw meat while still in a very immature condition.

Lot 2057 had a diet like that of Lot 2058 except that 15 per cent of dextrin was replaced by a like amount of lactose. There was

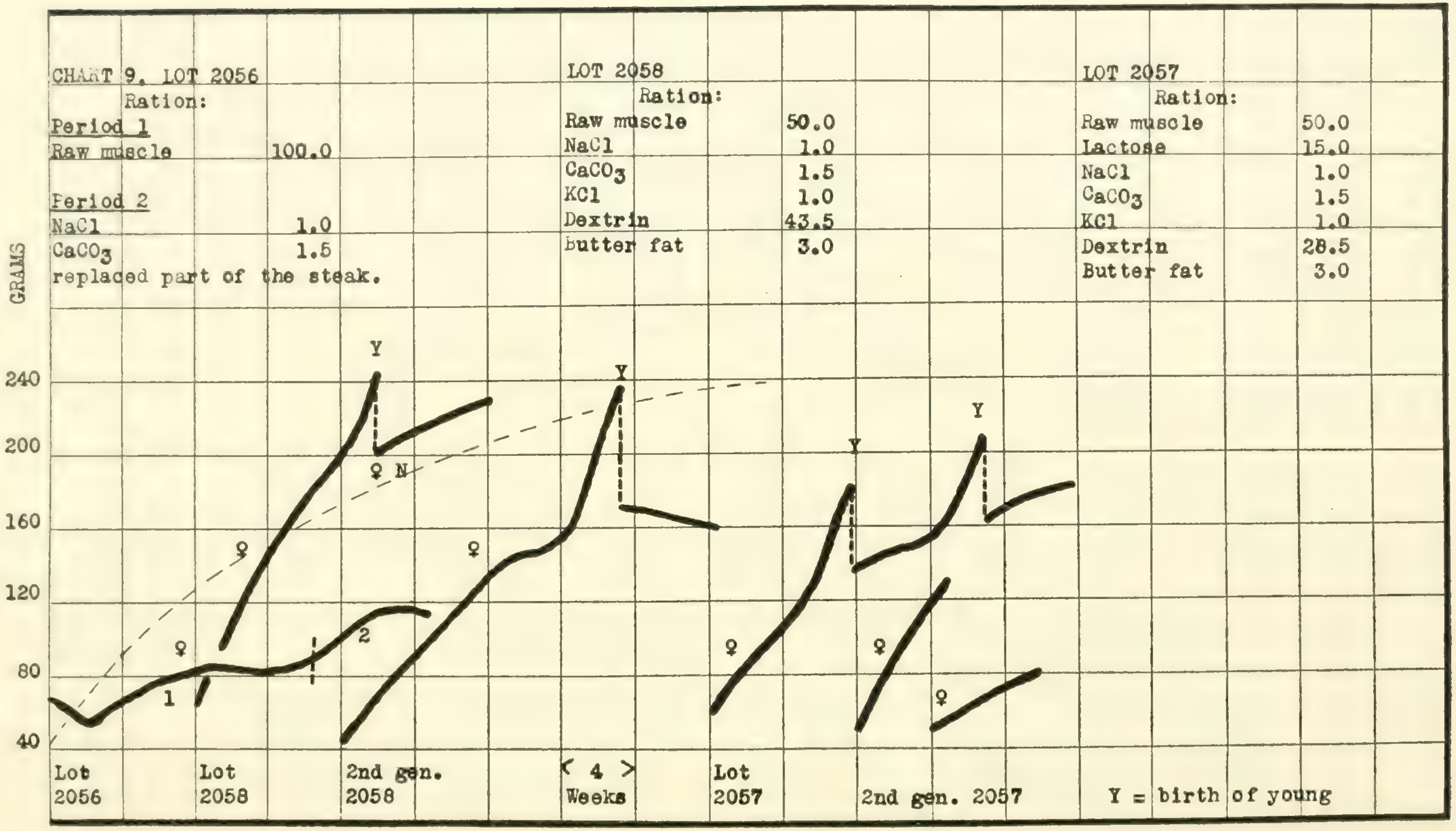


CHART 9. LOT 2056

Ration:

Period 1	
Raw muscle	100.0

Period 2	
NaCl	1.0
$CaCO_3$	1.5

replaced part of the steak.

LOT 2058

Ration:	
Raw muscle	50.0
NaCl	1.0
$CaCO_3$	1.5
KCl	1.0
Dextrin	43.5
Butter fat	3.0

LOT 2057

Ration:	
Raw muscle	50.0
Lactose	15.0
NaCl	1.0
$CaCO_3$	1.5
KCl	1.0
Dextrin	28.5
Butter fat	3.0

Y = birth of young

no noticeable benefit from this modification of the diet. There were two females in this group. They had collectively twenty-three young (four litters) of which twenty-two were weaned. No young have as yet been secured from any of the daughters. These records when compared with those of Chart 8 indicate that no noticeable difference exists in the nutritive value of raw and cooked steak. Although steak is very poor in water-soluble B, there was a sufficient amount of it in 50 per cent of cooked steak to permit young rats to grow up and rear young.

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SUPPLEMENTARY PROTEIN VALUES IN FOODS.

II. SUPPLEMENTARY DIETARY RELATIONS BETWEEN ANIMAL TISSUES AND CEREAL AND LEGUME SEEDS.

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(Received for publication, March 21, 1921.)

In a former publication[1] we have shown that the proteins of the navy bean do not markedly supplement those of the maize kernel so as to improve their quality for the support of growth. This means that the proteins of this cereal and legume seed are both deficient in some of the same indispensable amino-acids. Our earlier studies also indicated that the cereal proteins do not have much of a supplementary value among themselves.[2] In a later paper of this series we shall present more records bringing out the extent to which the proteins of the cereal grains and the legume seeds enhance each other's values for the nutrition of the rat. The data presented in the charts show the nutritive values of the protein mixtures resulting from combinations of the cereal grains and legume seeds with several representative animal tissues.

We recently pointed out the surprising fact that when muscle tissue, liver, or kidney, serves as the sole source of protein in the diet, all other factors in which have been made satisfactory by suitable additions, the quality of the amino-acid mixture for transformation into body proteins during growth is no better than that of proteins derived from grains.[3] It has always been accepted as a fact, hardly in need of experimental demonstration, that these animal proteins have exceptionally high biological values. Thomas came to this conclusion as the result of experimental data obtained on a full grown man as a subject. The periods of observation were very short, and a milk diet was used in the foreperiods which vitiated the results because of the supplementary effect on the foods being studied. Thomas' experimental data represent digestibility and absorbability of proteins rather than biological value for transformation into body tissue.[4]

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[1] McCollum, E. V., Simmonds, N., and Pitz, W., *J. Biol. Chem.*, 1917, xxix, 521.

[2] McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1919, xxxvii, 155.

[3] McCollum, E. V., Simmonds, N. and Parsons, H. T., *J. Biol. Chem.*, 1921, xlvii, 111.

TABLE I.

Chart.	Lot.	Source of protein.	Growth.	Fertility.	Mortality.
2	2193	⅔ wheat, ⅓ kidney.	Excellent.	High.	Medium.
	2192	⅔ soy beans, ⅓ kidney.	Fair.	Very low.	High.
3	2479	⅔ barley, ⅓ kidney.	Good.	High.	Medium.
4	2195	⅔ rolled oats, ⅓ kidney.	Fair.	Medium.	"
5	2194	⅔ maize, ⅓ kidney.	"	"	High.
	2191	⅔ navy beans, ⅓ kidney.	"	Low.	Low.
6	2190	⅔ peas, ⅓ kidney.	"	"	High.
	2196	⅔ rye, ⅓ "	Good.	Medium.	"

Of all the combinations of proteins from the two sources which we have studied, wheat and kidney proved to have the highest biological value. We have tested only one mixture of proteins from these sources; *viz.*, that in which a seed furnished two-thirds and kidney one-third of the total. Since in many cases growth was essentially normal, the basis of judgment in the comparison of the values of protein mixtures was the variation in fertility and infant mortality.

The combination of navy bean and kidney proteins forms a mixture having a higher biological value than any other animal tissue and legume seed which we have studied. Soy bean and kidney form a better protein mixture than soy bean with liver or soy bean and muscle.

The results of our studies show that the proteins of kidney, liver, and muscle have remarkable values for the enhancement of the proteins of some of the cereal grains, and improve the legume seed proteins to an appreciable degree. Kidney, liver, and muscle proteins have without exception supplemented those of the cereal grains more satisfactorily than they do those of the legume seeds. There are marked differences in the efficiency of the supplementary relations between kidney proteins and those of several cereal grains which make it possible for us to differentiate between their values in the case of the different combinations. The relative values of the several combinations of vegetable proteins with those of kidney with which we have experimented in this series may be illustrated by tabulating the seeds in the order of their efficiency for growth, when two-thirds of the protein is derived from the seed and one-third from kidney.

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[4] Thomas, K., *Arch. Physiol.*, 1909, 219.

Wheat—barley—rye—oat—maize—soy bean—pea—navy bean.

There is no marked differentiation between the kidney and oat as contrasted with the kidney and maize combinations. These are distinctly inferior to the combinations of kidney with barley, wheat, or rye. There is little difference in the biological values of the mixtures of proteins derived from peas or the two kinds of beans studied. These legume seeds combined with animal tissues are inferior to similar combinations of animal tissues with cereals. Table I shows in condensed form the effects of feeding kidney proteins as supplements to certain vegetable proteins on growth, fertility, and infant mortality.

Among the several combinations of cereal and legume seed proteins with muscle tissue which we have studied, wheat is decidedly the best. Growth and fertility were remarkable, but the infant mortality was high. Muscle tissue is distinctly less effective than kidney as a supplement to the proteins of the seeds used, except in the case of wheat. The following order represents the biological values of combinations of seed with muscle in which the seed furnished two-thirds of the total protein and the muscle one-third. The values are arranged in a descending scale.

Wheat—oat—barley—maize—pea—navy bean—soy bean.

It is not possible to differentiate from our data between the values of navy bean and soy bean proteins combined with muscle. Table II shows in condensed form the effects on growth, fertility, and infant mortality of feeding muscle proteins as supplements to the proteins of several seeds.

Liver proteins supplement those of the cereal grains and legume seeds in degrees which differ sufficiently to make possible rather decided contrasts in several cases. In general, liver proteins enhance those of the seeds somewhat better than do those of muscle, with the single exception of the combination, wheat and muscle. The seeds which we have studied may be arranged in the following order to illustrate the supplementary efficiency of liver proteins for each, the best combination being on the left and decreasing from left to right.

Barley—rye—wheat—oat—maize.

TABLE II.

Chart.	Lot.	Source of protein.	Growth.	Fertility.	Mortality.
7	2186	$\frac{2}{3}$ wheat, $\frac{1}{3}$ muscle.	Excellent.	High.	High.
8	2189	$\frac{2}{3}$ rye, $\frac{1}{3}$ "	Very good.	"	"
	2184	$\frac{2}{3}$ navy beans, $\frac{1}{3}$ muscle.	Poor.	Low.	"
9	2188	$\frac{2}{3}$ rolled oats, $\frac{1}{3}$ "	Good.	High.	"
	2477	$\frac{2}{3}$ barley, $\frac{1}{3}$ muscle.	"	Low.	"
10	2187	$\frac{2}{3}$ maize, $\frac{1}{3}$ "	Fair.	"	"
	2183	$\frac{2}{3}$ peas, $\frac{1}{3}$ "	"	"	"
	2185	$\frac{2}{3}$ soy beans, $\frac{1}{3}$ muscle.	"	"	"

Wheat and muscle gave an excellent growth curve and high fertility, but apparently because of a slight inferiority of this protein mixture as compared with certain others, the infant mortality was high. Wheat and muscle appear to form a protein mixture superior to rye and muscle. The growth curves of rats confined to a protein supply derived from rolled oats and muscle were better than the curves of those fed rolled oats and kidney. The latter, however, were more successful in fertility and in rearing young than the former.

It is not possible from our data to decide between the values of the combinations of liver proteins with those of the legume seeds, peas, navy beans, and soy beans. A study of the records presented here, showing the effects of seed and meat diets, supplemented with respect to all factors other than protein, on the life histories of individual animals and on the family histories of successive generations confined to monotonous food mixtures, cannot fail to impress one with the fact that observations on the growth curves of a group of animals to maturity do not afford sufficient evidence to enable one to arrive at safe deductions concerning the quality of an experimental diet. Table III shows the effect on growth, fertility, and infant mortality, of feeding liver proteins as supplements to the proteins of several seeds.

There are many who believe that if the diet affords sufficient calories and considerable variety, satisfactory nutrition will be assured. This may be far from true. So long as the selection does not include a variety of types of foods of unlike dietary values variety does not insure safety in any great degree. No matter how many cereals, tubers, and muscle meats such as steak, ham, chops, roasts, etc., may be taken, the diet will prove to be inadequate. The inclusion of milk or leafy vegetables goes far toward making good deficiencies of such a diet in inorganic constituents and fat-soluble A. Fresh fruits and uncooked salad vegetables have a unique place in the diet as sources of the antiscorbutic factor water-soluble C.

TABLE III.

Chart.	Lot.	Source of protein.	Growth.	Fertility.	Mortality.
11	2478	$\frac{2}{3}$ barley, $\frac{1}{3}$ liver.	Good.	High.	Medium.
	2178	$\frac{2}{3}$ soy beans, $\frac{1}{3}$ liver.	Poor.	Low.	High.
12	2180	$\frac{2}{3}$ maize, $\frac{1}{3}$ liver.	Fair.	Medium.	“
13	2181	$\frac{2}{3}$ rolled oats, $\frac{1}{3}$ liver.	Good.	High.	“
14	2182	$\frac{2}{3}$ rye, $\frac{1}{3}$ liver.	Very good.	“	“
	2176	$\frac{2}{3}$ peas, $\frac{1}{3}$ “	Fair.	Low.	“
15	2179	$\frac{2}{3}$ wheat, $\frac{1}{3}$ “	Good.	High.	“
	2177	$\frac{2}{3}$ navy beans, $\frac{1}{3}$ liver.	Fair.	Low.	“

Rolled oats and liver formed a better protein mixture than rolled oats with kidney or muscle. This could not be discerned from an inspection of the growth curves or the records of fertility, but came to light through the comparison of the infant mortality in the three groups.

Barley and liver made a better protein mixture than barley and muscle, but not quite so good as barley and kidney. Rye and liver also proved superior as a source of protein to mixtures of this cereal with either muscle or kidney.

Navy beans with liver form a better protein mixture than combinations of this seed with muscle, but not quite so good as with kidney.

The substitution at frequent intervals of ham for chops will not tend to insure safety in nutrition. So large a part of the food supply of man in the temperate and warmer regions of the world is derived from cereals and other seed grains, tubers, and edible roots, that in seeking variety in food attention should be fixed upon the importance of milk and the leafy vegetables suitable for greens, salads, etc., since these are so constituted as to correct the deficiencies of cereals, tubers, and roots.[5]

In examining the records of the experimental animals described in this paper it should be kept in mind that in studying the combinations of proteins from animal and vegetable sources, we have in all cases added butter fat to furnish fat-soluble A and the inorganic salts necessary to correct the mineral deficiencies. The results do not, therefore, represent the supplementary effect of one of these foodstuffs for another except in respect to the protein factor.

The results of these studies form a demonstration of the value of animal tissues as constituents of the diet, when the latter contains as much cereal products as is usually the case in America and Europe at the present time. At the same time it must be remembered that the animal tissues are by no means complete supplements for a cereal and legume seed diet.

SUMMARY.

1. The proteins of kidney, liver, and muscle are remarkably effective as supplements for the proteins of cereals. There are demonstrable differences in the extent to which these animal tissues enhance the values of certain of the cereals. Thus, kidney, liver, and muscle are about equally effective as supplements to wheat. Maize proteins are less effectively supplemented by kidney, liver, or muscle than wheat, barley, or rye.

2. The three animal tissue proteins studied were found to have a supplementary relation to pea, navy bean, or soy bean proteins, but these combinations are very inferior to similar combinations of animal tissues with cereal grains as a source of protein.

3. Our results demonstrate that the proteins of kidney, liver, or muscle are more valuable for transformation into body tissues when combined with cereal proteins, than when each is fed as the sole source of amino-acids in the diet.[3]

4. Either muscle or glandular substance fails to supplement effectively the mineral deficiencies of cereals or legume seeds. The glandular organs are good sources of fat-soluble A.

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[5] McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1919, xxxviii, 113.

Chart 1.—These curves show typical records of growth and reproduction of rats fed from an early age on diets in which the sole source of protein was a cereal or legume seed. The amount of seeds was adjusted in each case so as to give the diets a protein content of 9 per cent. Such additions of butter fat and inorganic salts were made in each case as to correct dietary deficiencies other than protein of these seeds.

The list of cereals here discussed includes wheat, maize, rolled oats, barley, and the legumes, peas, navy beans, and soy beans.

Among these seeds wheat stands first in quality of protein when fed as the sole source of this factor. Oat and maize proteins have approximately the same value, but we have gained the impression that oat proteins are slightly superior. None of the legume seeds is at all satisfactory as the sole source of protein in the diet at the plane of intake of 9 per cent of the food mixture. This fact is brought out by the curves of Lots 2376, 2510, and 2368. On adding casein in a second period growth was accelerated in each case.

No young have been secured on diets of this type in which proteins were derived entirely from a legume seed or from maize. The fertility of rats fed oats to furnish 9 per cent of protein was so far below that of animals fed wheat protein in similar amounts as to indicate that the latter were in a distinctly better state of well being. Even in the case of the wheat diet the protein was not sufficiently good to permit of much success in rearing young. A few young could be reared by mothers which had grown up and were maintained on the oat diet containing 9 per cent of protein.

These curves are presented as a basis of comparison for the records in the remaining charts described in this paper. These derive their content of protein (9 per cent) in each case from two sources. The object in each case is to compare the biological value of mixtures of proteins in stated proportions as compared with the values of each source when fed alone.

Chart 2.—Lot 2193 was fed a diet from soon after weaning, which derived its protein content of 9 per cent from wheat and cooked dry kidney. All other factors in the diet were adequately supplemented. The wheat furnished 6 per cent and the kidney 3 per cent of the protein of the diet. Growth was normal in this group. The state of nutrition of these animals was much superior

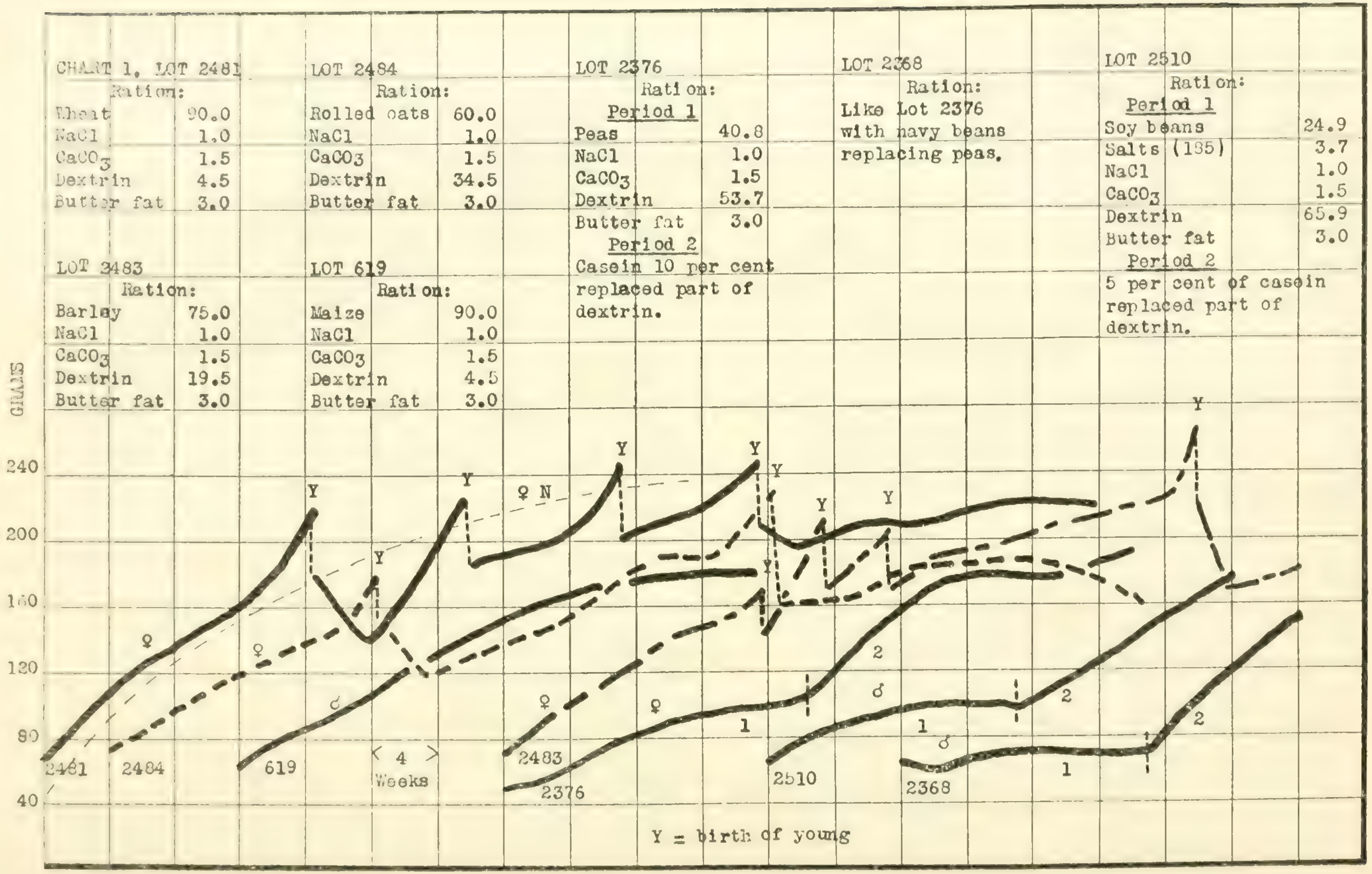


CHART 1. LOT 2481

Ration:

Wheat	90.0
NaCl	1.0
$CaCO_3$	1.5
Dextrin	4.5
Butter fat	3.0

LOT 2483

Ration:

Barley	75.0
NaCl	1.0
$CaCO_3$	1.5
Dextrin	19.5
Butter fat	3.0

LOT 2484

Ration:

Rolled oats	60.0
NaCl	1.0
$CaCO_3$	1.5
Dextrin	34.5
Butter fat	3.0

LOT 619

Ration:

Maize	90.0
NaCl	1.0
$CaCO_3$	1.5
Dextrin	4.5
Butter fat	3.0

LOT 2376

Ration:

Period 1

Peas	40.8
NaCl	1.0
$CaCO_3$	1.5
Dextrin	53.7
Butter fat	3.0

Period 2

Casein 10 per cent replaced part of dextrin.

LOT 2368

Ration:

Like Lot 2376 with navy beans replacing peas.

LOT 2510

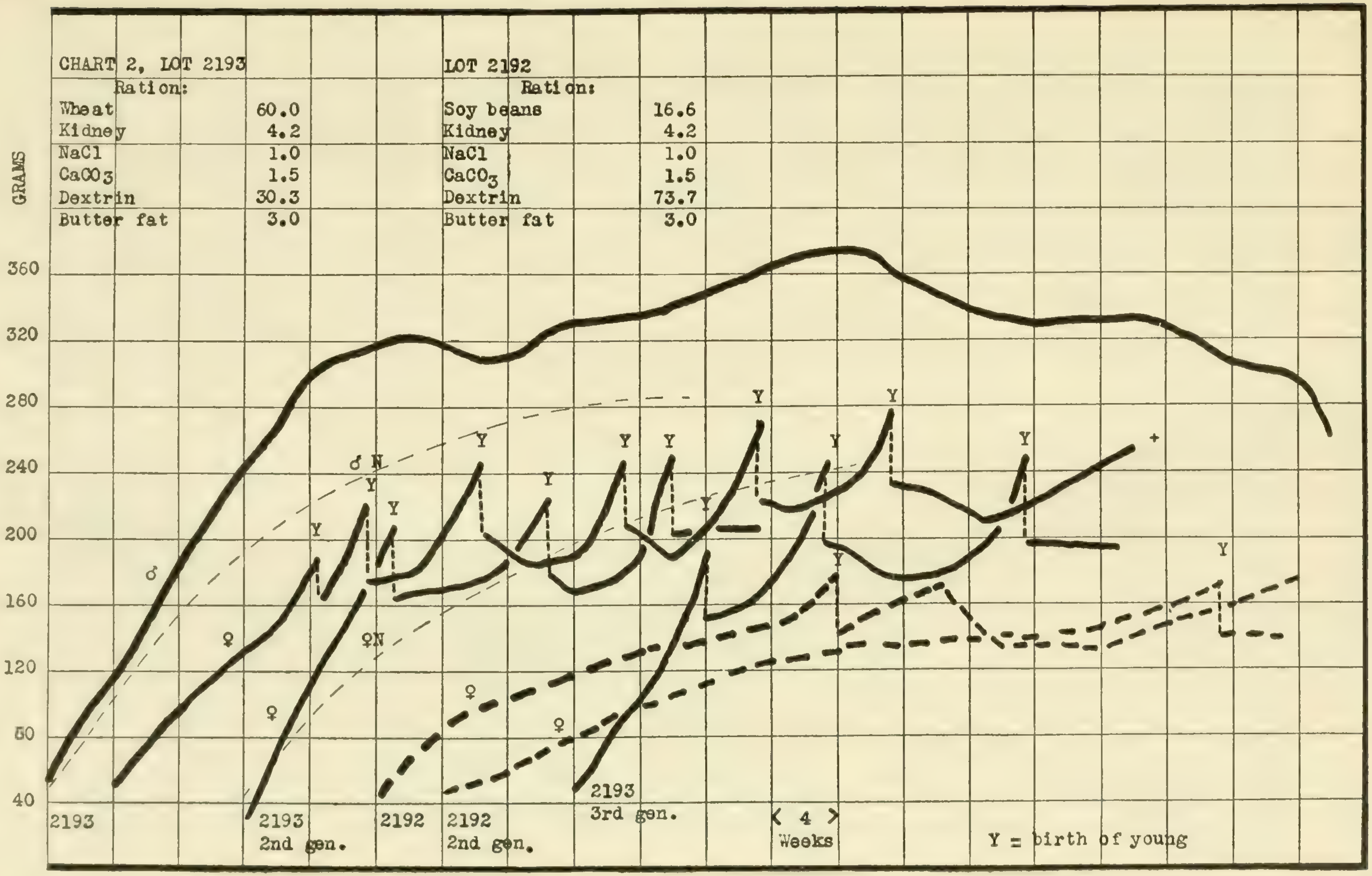
Ration:

Period 1

Soy beans	24.9
Salts (185)	3.7
NaCl	1.0
$CaCO_3$	1.5
Dextrin	65.9
Butter fat	3.0

Period 2

5 per cent of casein replaced part of dextrin.

CHART 2, LOT 2193
Ration:
Wheat 60.0
Kidney 4.2
NaCl 1.0
CaCO3 1.5
Dextrin 30.3
Butter fat 3.0
LOT 2192
Ration:
Soy beans 16.6
Kidney 4.2
NaCl 1.0
CaCO3 1.5
Dextrin 73.7
Butter fat 3.0
GRAMS
360
320
280
240
200
160
120
80
40
♂
♀
♀
♂N
♀N
♀
♀
Y
2193
2193
2nd gen.
2192
2192
2nd gen.
2193
3rd gen.
‹ 4 ›
Weeks
Y = birth of young
+

to those fed wheat as the sole source of protein when the content of the latter was limited to 9 per cent. Those rats which grew up on 9 per cent of wheat protein (Lot 2481, Chart 1) were as old looking at 14 months as were those in Lot 2193 at 18 to 20 months. Indeed, the animals of Lot 2193 were in such good condition that they compared favorably in appearance and in fertility with other experimental groups fed much higher protein intakes.

There were two females in this group. They had collectively seventy-nine young (eleven litters) and weaned seventy of them. On a diet comparable in all respects but with all its 9 per cent of protein derived from wheat we observed 50 per cent mortality of the young. A somewhat higher protein content in the diet of Lot 2193 would apparently have shortened the nursing periods, which were longer than normal, and would have lowered the infant mortality. Four females in the second generation had seventy-five young (twelve litters) and weaned forty-nine of them. Two third generation females had forty-nine young (eight litters) and weaned thirty-six. The mortality of the young in this group was due entirely to destruction by the mothers. Frequently the young were bitten and injured or killed when approaching the end of the nursing period.

One cannot say that animals, which had a diet containing enough protein to enable them to grow at the normal rate to maturity and to be as fertile as were these, were suffering from protein starvation. Yet it was due to the low protein intake (of the quality here used) which exerted a peculiar influence on the psychological reactions of the mothers and made them attack their young even after they were 2 or 3 weeks old. In rats in our colony which have highly satisfactory diets it is extremely rare that a mother attacks her offspring or indeed those of another. We not infrequently isolate among our stock animals two or three pregnant females in the same cage. They then care for their young in a common nest and nurse their own young and those of the other mothers promiscuously and without perversion of the maternal instinct.

Lot 2192 was fed a diet in which the protein was furnished by soy beans and beef kidney. The diet contained 9 per cent of total protein, two-thirds of which came from soy beans and one-third from kidney. All the other factors were satisfactorily supplemented. Growth was slow and the animals never reached full adult size. Their growth was much better, however, than that of rats restricted to the same amount of protein entirely from soy beans (Chart 1, Lot 2510).

Two females grew up on this diet. One remained sterile. The other had nine young (two litters) and weaned three. Two second generation females remained sterile. The animals in this group were very old looking at 15 months of age. It is evident that 9 per cent of protein from these sources is not satisfactory for normal milk production.

Chart 3.—Lot 2479 was fed a diet in which the protein was derived from barley and kidney. The barley furnished two-thirds and the kidney one-third of the total protein. The growth was more satisfactory than any we have observed in rats fed 9 per cent of barley protein only (see Chart 1, Lot 2483).

Two females which grew up on this diet had collectively forty-five young (seven litters) and weaned thirty-one. One female in the second generation had nineteen young (three litters) and weaned eighteen. Two third generation females had forty-three young (seven litters) and weaned nineteen. One fourth generation female was restricted to the diet but she failed early and died at the age of 110 days. These animals were in good condition at the age of 15 months, when they were discarded.

Chart 4.—Lot 2195 was fed a diet in which the protein was furnished entirely by rolled oats and kidney The oats furnished two-thirds and the kidney one-third of the total protein. Their records were very much better than those of Lot 2484 (Chart 1). This is shown especially in the fertility and infant mortality of the two groups. Lot 2195 was somewhat undersized but of good appearance. They were not in such good condition as those which grew up on the wheat and kidney diet (Lot 2193, Chart 2).

Two females grew up on this diet. One of these had twenty young (four litters) and weaned ten. The other died soon after giving birth to her first litter of six young. Two second generation females had thirty-seven young (six litters) and weaned twenty-three. One third generation female had one litter of five and weaned them all. These young were in good condition, but the nursing periods were long. The animals in this group were old looking at about 16 months.

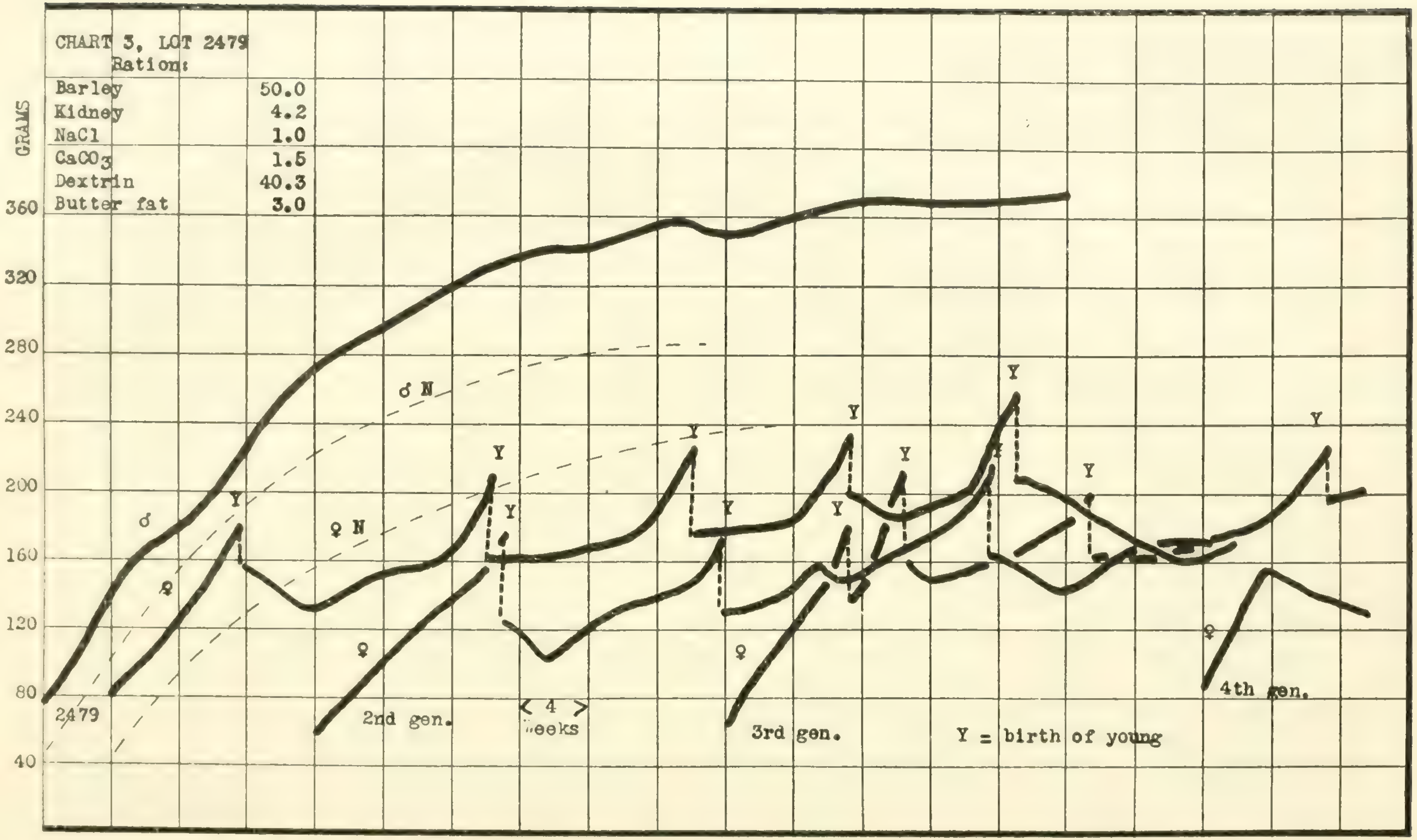


CHART 3, LOT 2479

Ration:

Barley	50.0
Kidney	4.2
NaCl	1.0
$CaCO_3$	1.5
Dextrin	40.3
Butter fat	3.0

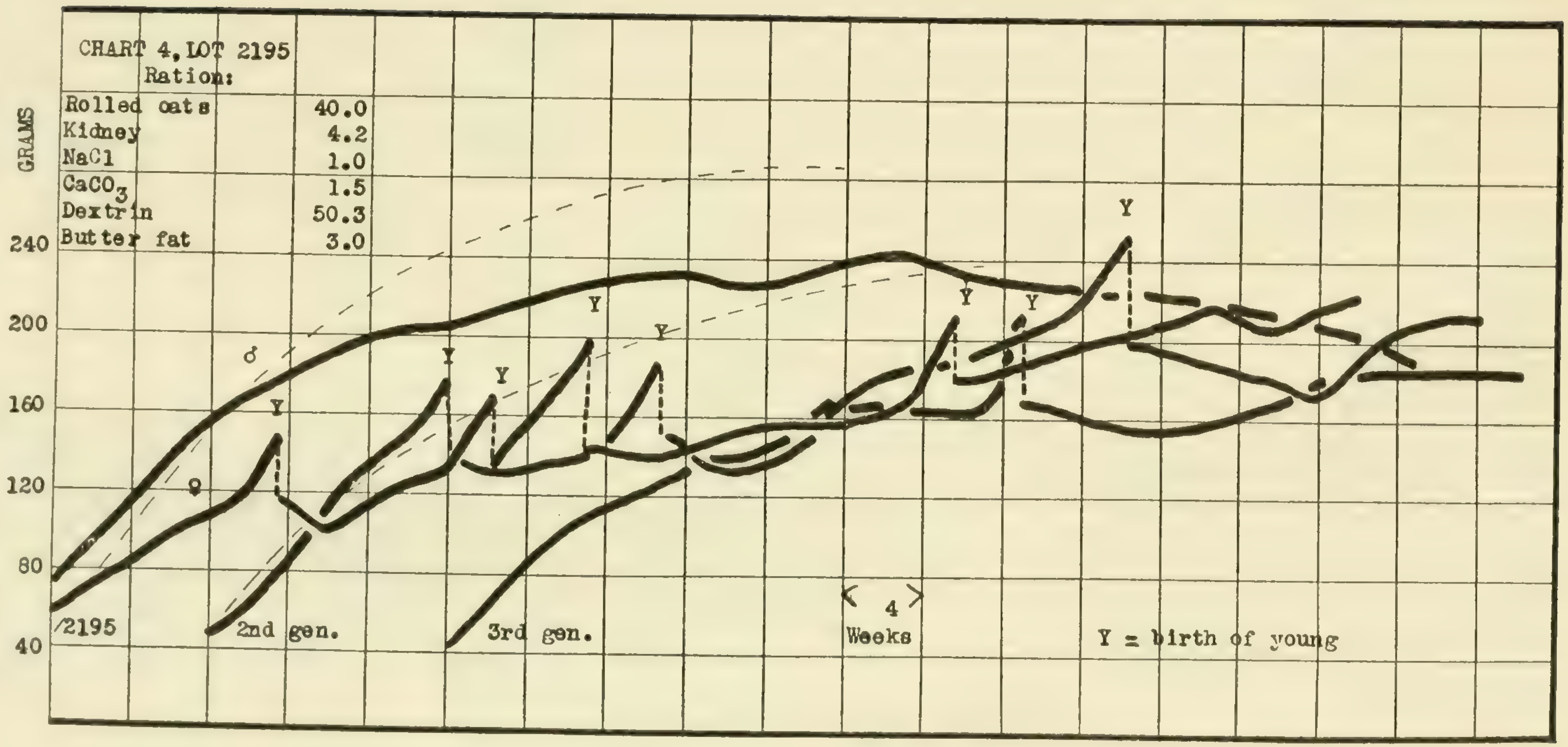


CHART 4, LOT 2195
Ration:
Rolled oats 40.0
Kidney 4.2
NaCl 1.0
CaCO$_3$ 1.5
Dextrin 50.3
Butter fat 3.0
GRAMS
240
200
160
120
80
40
♂
♀
Y
2195
2nd gen.
3rd gen.
< 4 >
Weeks
Y = birth of young

Chart 5.—Lot 2194 was fed a diet, the protein of which was derived entirely from maize and kidney. The maize furnished two-thirds of the total protein and the kidney one-third. All other factors were satisfactorily supplemented. The growth curves were much better than were those of Lot 619 (Chart 1), which had a diet containing 9 per cent of maize protein alone. The animals of Lot 2194 on the maize and kidney proteins were never in good condition, but did not look very aged until about 18 months. Three females which grew up on this diet had thirty-one young (six litters) and weaned fifteen. They were only in fair condition and were small for their ages. One second generation female grew up on the diet and had fifteen young (three litters). She weaned four of her third litter. The other young she destroyed soon after they were born.

Lot 2191 was fed a diet containing 9 per cent of protein, two-thirds of which was furnished by navy beans and one-third by kidney. All factors other than protein were satisfactorily supplemented. A diet exactly similar but with 9 per cent of protein derived solely from navy beans supports almost no growth (Lot 2368, Chart 1). The combination of navy beans and kidney is therefore vastly superior to bean protein alone. These animals looked very old at about 12 months.

One female in this group had one litter of five young and weaned four, but they were very small. At 42 days of age the four weighed but 147 gm. A litter of four nursing a normally fed mother should reach a weight of about 350 gm. at this age. One other female in this group remained sterile. Two second generation females were restricted to the diet but neither one had any young although their growth curves were much better than those of rats confined to 9 per cent of navy bean protein alone (Lot 2368, Chart 1).

Chart 6.—Lot 2190 was fed a diet containing 9 per cent of protein, two-thirds of which was furnished by peas and one-third by kidney. All other factors were satisfactorily supplemented. The animals grew much better than a group confined to a similar diet with 9 per cent of pea protein as its sole source of nitrogen (Lot 2376, Chart 1). This shows that there is a greatly heightened value in the combination of proteins used in this diet as compared with pea protein alone. The rats fed this ration were somewhat

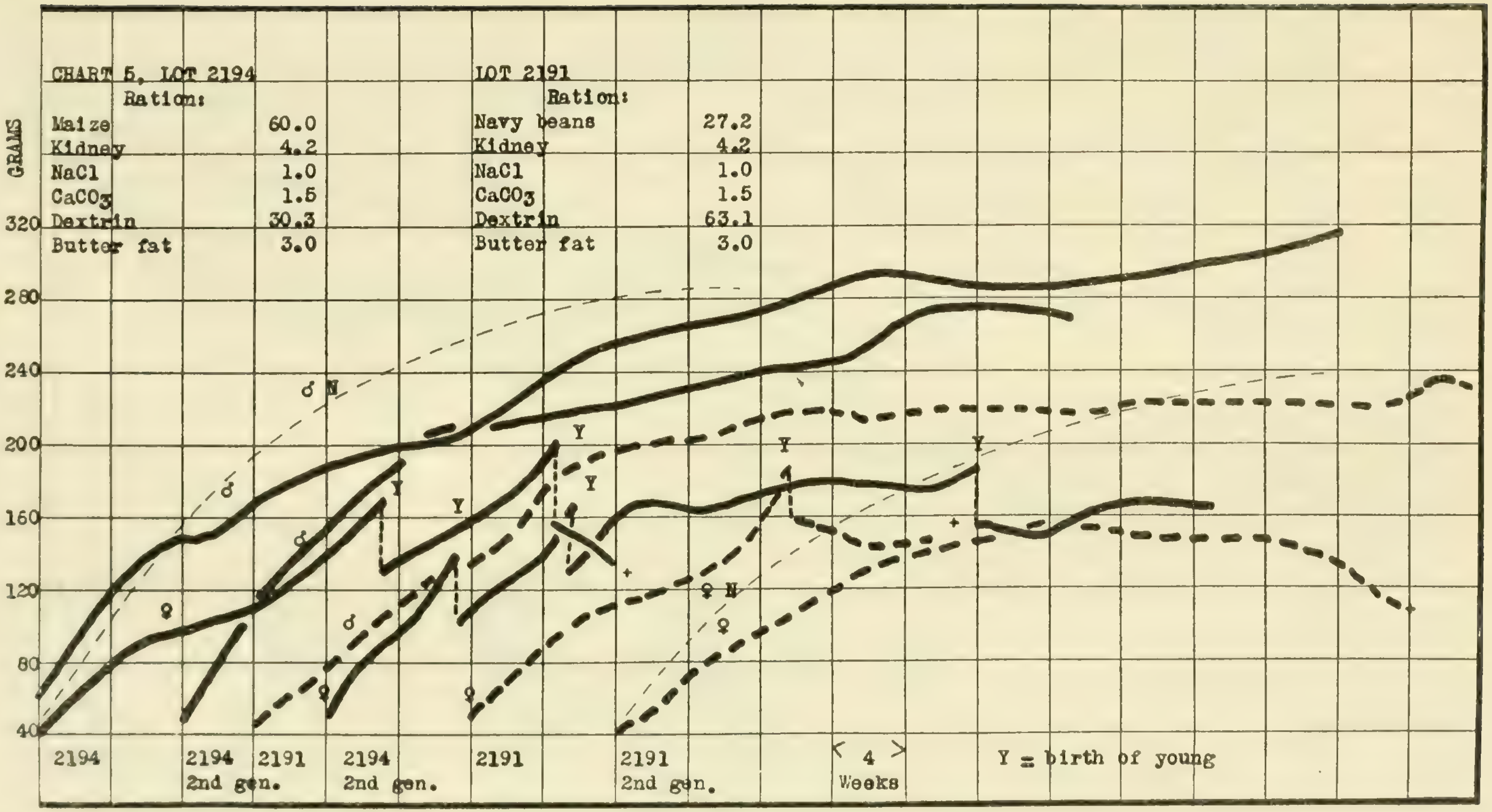


CHART 5, LOT 2194
Ration:
Maize 60.0
Kidney 4.2
NaCl 1.0
CaCO3 1.5
Dextrin 30.3
Butter fat 3.0
LOT 2191
Ration:
Navy beans 27.2
Kidney 4.2
NaCl 1.0
CaCO3 1.5
Dextrin 63.1
Butter fat 3.0
GRAMS
320
280
240
200
160
120
80
40
♂ N
♂
♀
♂
Y
Y
♂
♀
♀
Y
Y
Y
♀ N
♀
Y
2194
2194
2nd gen.
2191
2194
2nd gen.
2191
2191
2nd gen.
< 4 >
Weeks
Y = birth of young

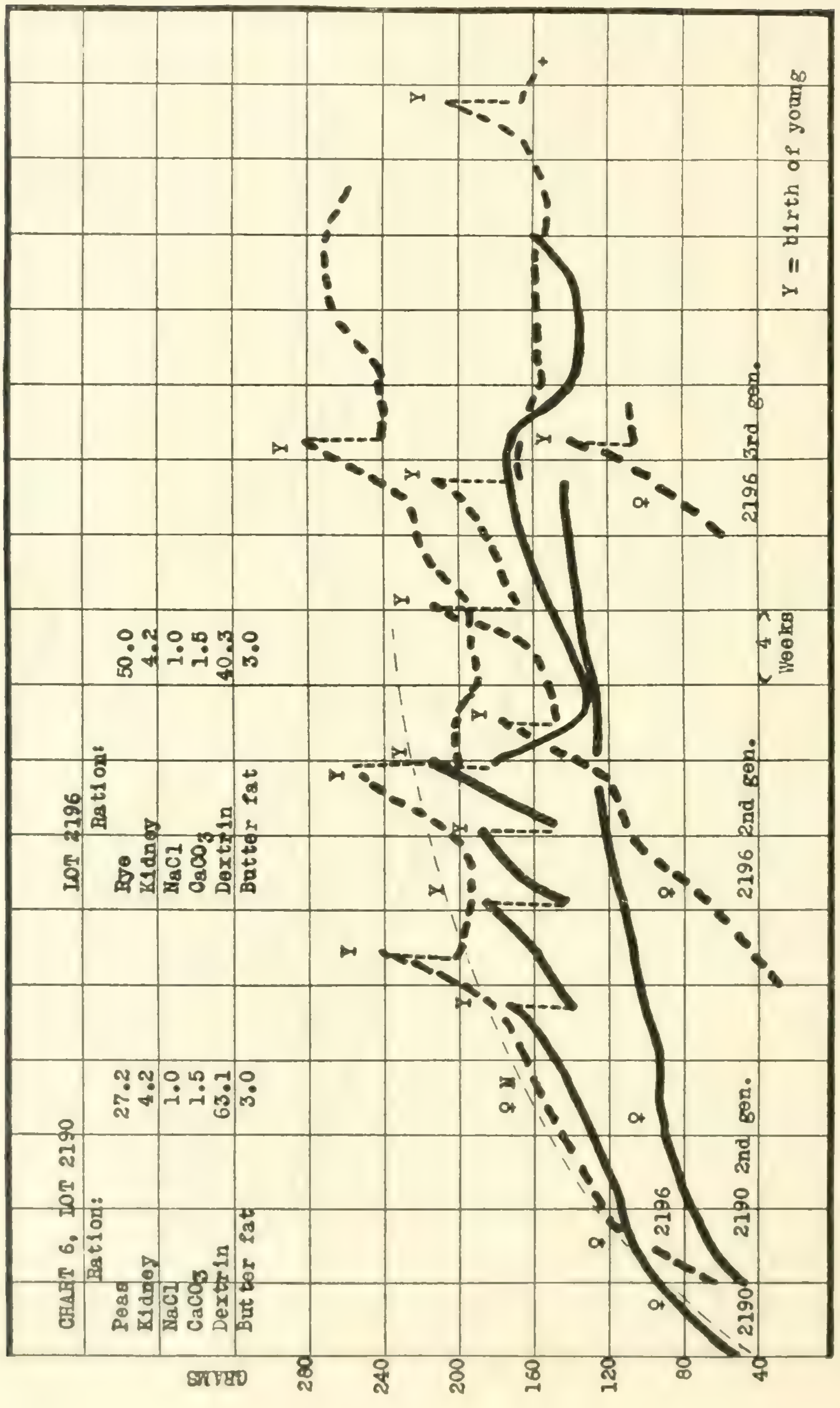


CHART 6, LOT 2190
Ration:
Peas 27.2
Kidney 4.2
NaCl 1.0
$CaCO_3$ 1.5
Dextrin 63.1
Butter fat 3.0
LOT 2196
Ration:
Rye 50.0
Kidney 4.2
NaCl 1.0
$CaCO_3$ 1.5
Dextrin 40.3
Butter fat 3.0
GRAMS
280
240
200
160
120
80
40
2190
2196
2190 2nd gen.
2196 2nd gen.
2196 3rd gen.
♀
♀ M
Y
< 4 > Weeks
Y = birth of young

undersized, but appeared fairly well nourished in early life. They appeared senile when about 12 months of age.

One female in this group had twenty-four young (four litters) and weaned four of her fourth litter. The other young were destroyed by the mother while very young. Another female remained sterile. Three second generation females were confined to the diet until they were 12 months old, but none of them had any young.

Lot 2196 derived its sole protein from rye and kidney. The rye furnished two-thirds and the kidney one-third of the total. Except for the amount and quality of the protein the diet was well constituted. Although the growth of these animals was excellent and the fertility fairly high, the mortality of the young was very high. These rats were in good condition in early life but looked very old at about 16 to 18 months.

Five females on this diet had collectively seventy-three young (fourteen litters) and weaned thirty-one. Many of these were destroyed by the mothers soon after birth. One female died after the first litter, and a second died after her second litter from undetermined causes. Two second generation females had thirty-one young (seven litters) and weaned nine. One third generation female had a litter of six.

Our investigations show that cereal grain proteins are distinctly better supplemented by kidney proteins than are those of the legume seeds. The order of effectiveness in this supplementary relation is as follows:

Wheat—barley—rye—oat—maize.

There is no marked difference in the value of peas, soy beans, and navy beans in their supplementary relation to kidney proteins.

Chart 7.—Lot 2186 was fed a diet comparable to those discussed in Charts 2 to 6 inclusive, except that muscle tissue (beefsteak) replaced kidney as a supplemental source of protein for a seed. This diet contained 9 per cent of protein, two-thirds of which was furnished by wheat and one-third by beef muscle. Deficiencies in inorganic salts and fat-soluble A were made good by suitable additions.

The growth curves of this group, considering that the food mixture contained but 9 per cent of protein, are remarkable. All the

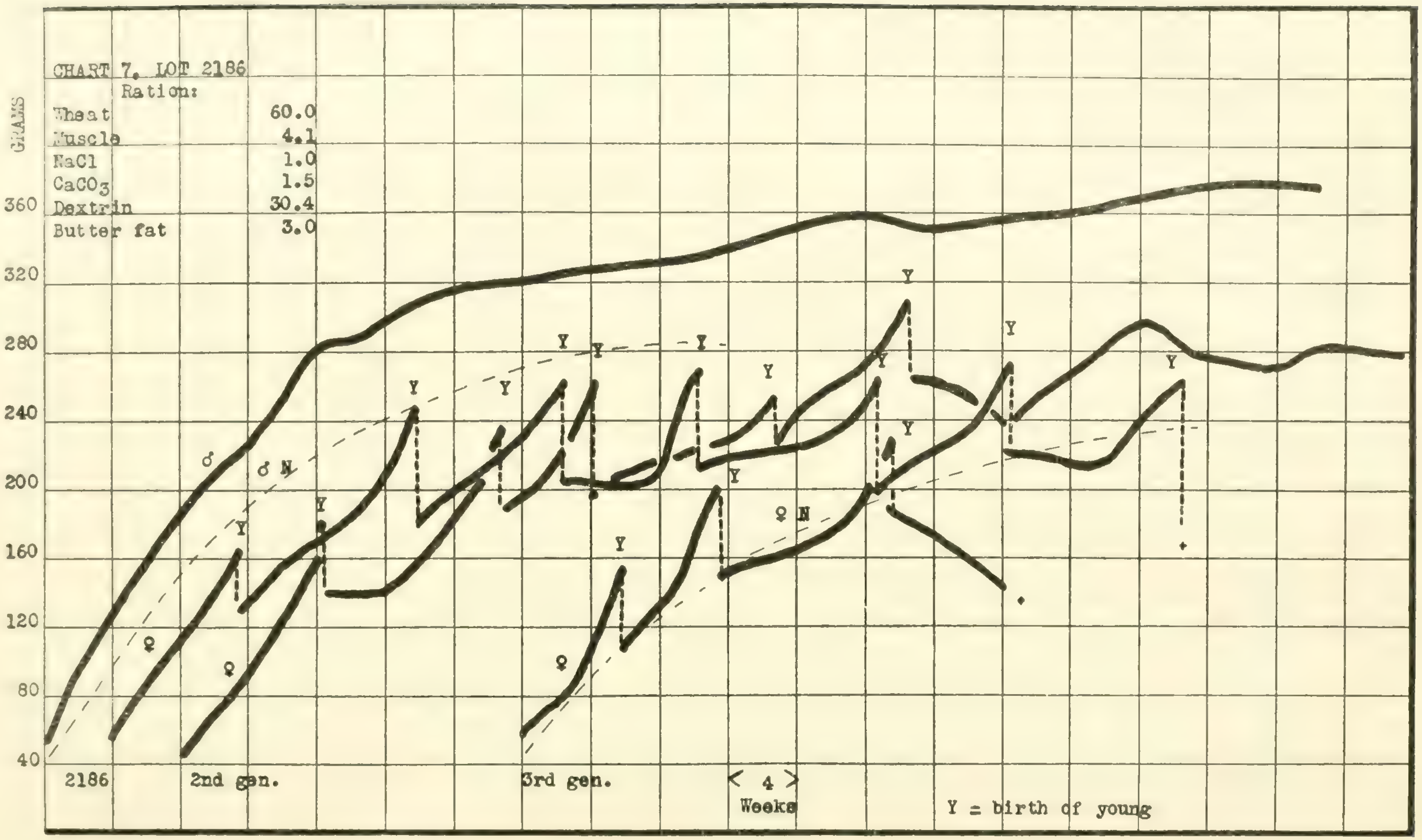


CHART 7. LOT 2186
Ration:
Wheat 60.0
Muscle 4.1
NaCl 1.0
CaCO3 1.5
Dextrin 30.4
Butter fat 3.0
GRAMS
360
320
280
240
200
160
120
80
40
2186
2nd gen.
3rd gen.
< 4 >
Weeks
Y = birth of young
♂
♂ N
♀
♀
♀
♀ N
Y

animals grew at a rapid rate to a large size. This can be interpreted only to mean that there is an excellent supplementary relation between the proteins of the wheat kernel and those of muscle tissue. This was likewise true of wheat supplemented with kidney (Lot 2193, Chart 2). The span of life was, however, somewhat short. The animals looked old at 16 to 17 months, whereas they remain vigorous up to 2 years at least when the nutrition is highly satisfactory. That they were not in this optimal condition, notwithstanding their large size and splendid fertility, is shown by the high mortality among their young. Although the biological value of the protein was high and the plane of intake sufficient to support growth and reproduction, the amount of protein of this quality was not good enough to maintain physiological well being to an advanced age or to suffice for the nutrition of the nursing young.

Two females grew up on this diet. They had collectively sixty young (eight litters) of which only twenty-nine were weaned. Two females of the second generation had sixty-five young (eleven litters) and weaned twenty-three. One third generation female had nineteen young (three litters) and weaned only three. Many of the young were destroyed shortly after birth, but those which were weaned were in good condition. In several instances the mothers reared large litters (nine young; seven young). This shows that even 9 per cent of protein of the excellent quality formed by wheat and muscle in the proportions here used sufficed for milk production even when the litters were large. We differentiate between high infant mortality due to infanticide and that due to undernutrition of the nursing young. It is most remarkable that rats on certain types of faulty diets should fail to exhibit the normal maternal instinct, which is very strong in this species.

Chart 8.—Lot 2189 was fed 9 per cent of protein in the form of rye and muscle. The cereal furnished two-thirds and the muscle one-third. The condition of these animals was not so good as that of Lot 2186 (Chart 7) fed a similar diet with the protein derived from wheat and muscle. They grew senile at about the same age as did Lot 2186.

Two females had collectively thirty-nine young (seven litters) and weaned fifteen. Two females of the second generation had forty-four young (eight litters) and weaned fifteen. Two third

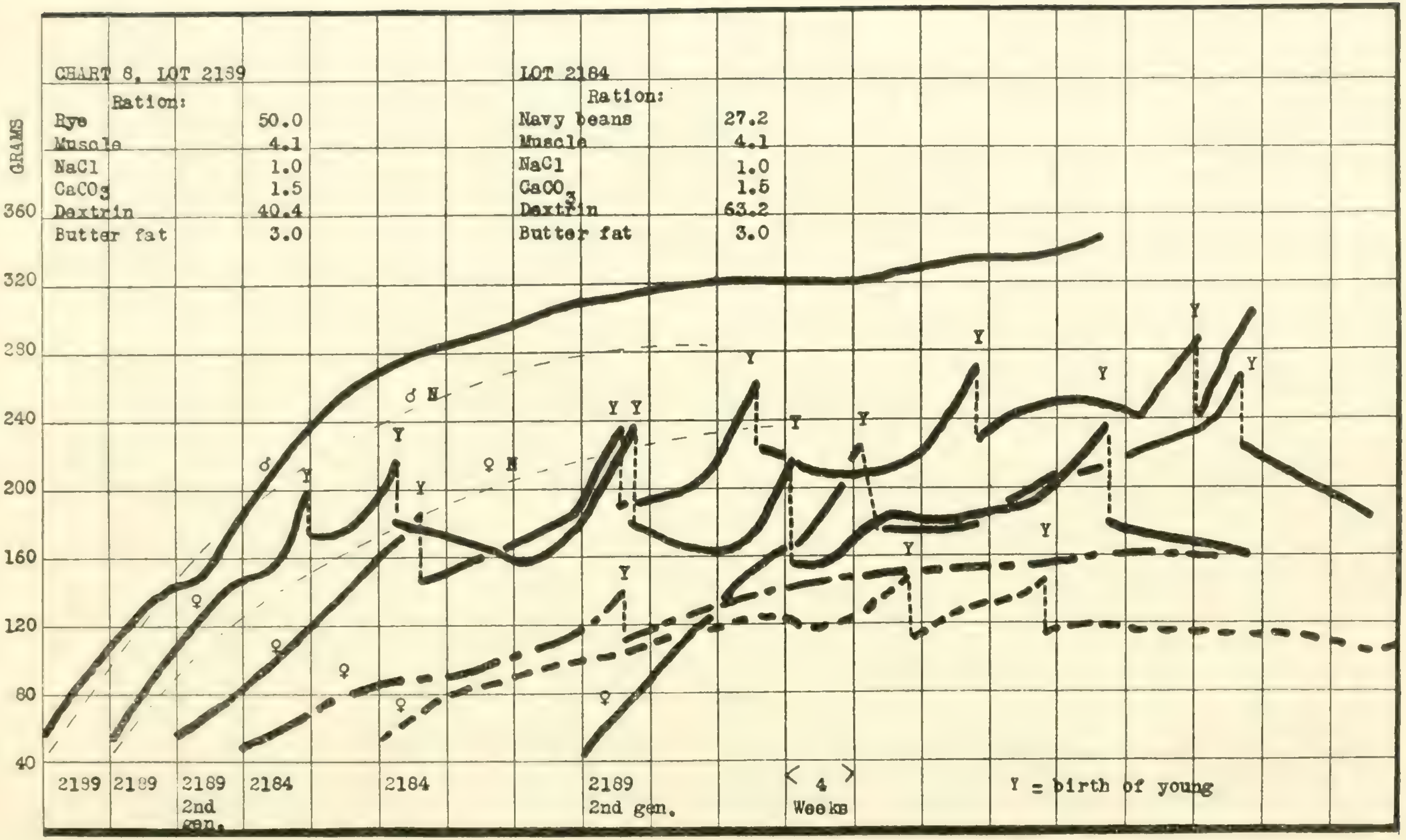


CHART 8, LOT 2189

Ration:	
Rye	50.0
Muscle	4.1
NaCl	1.0
$CaCO_3$	1.5
Dextrin	40.4
Butter fat	3.0

LOT 2184

Ration:	
Navy beans	27.2
Muscle	4.1
NaCl	1.0
$CaCO_3$	1.5
Dextrin	63.2
Butter fat	3.0

generation females, had each a litter (eleven young) and weaned six. One third generation female remained sterile. The young of all generations always presented an inferior appearance. The nursing periods were very long and in some cases the young remained undersized. In one instance two young which survived to the 41st day were still nursing and weighed but 60 gm. together.

Lot 2184 was fed 9 per cent of protein in the form of navy beans (6 per cent) and muscle tissue (3 per cent). Other factors than protein were satisfactory. These rats grew poorly and their fertility was low. The growth on this diet was better than we have seen on 9 per cent of navy bean protein alone. They were very old looking at 15 months.

Two females had collectively ten young (three litters) and destroyed them all shortly after birth.

Chart 9.—Lot 2188 was fed a diet in which the total protein content was 9 per cent, two-thirds of which was furnished by rolled oats and one-third by muscle tissue. The growth curves were much better than on 9 per cent of oat protein alone (Chart 1, Lot 2484), and the animals remained longer in a good condition. They were old looking at 15 to 16 months of age. Oat and muscle proteins are not of so good quality as a similar mixture derived from wheat and muscle, but of about the same value as a mixture derived from maize and muscle. This is shown by a comparison of Lot 2188, Chart 9, with Lot 2186, Chart 7, and Lot 2187, Chart 10, both with respect to the growth curves and reproduction records.

There were two females in this group. They had seven litters (thirty-seven young) and killed them all. The young were destroyed by the mothers soon after birth. Another litter was born but destroyed before the number was ascertained.

Lot 2477 was restricted to a diet containing 9 per cent of protein. Barley furnished 6 per cent and muscle tissue 3 per cent. All other factors were made satisfactory by suitable additions. Their growth and ability to rear young were inferior to Lot 2186 (Chart 7) which was fed wheat and muscle tissue. They were very old looking at 14 months of age.

Three females in this group had collectively seventy-five young (seven litters) and weaned only two. After these two were weaned

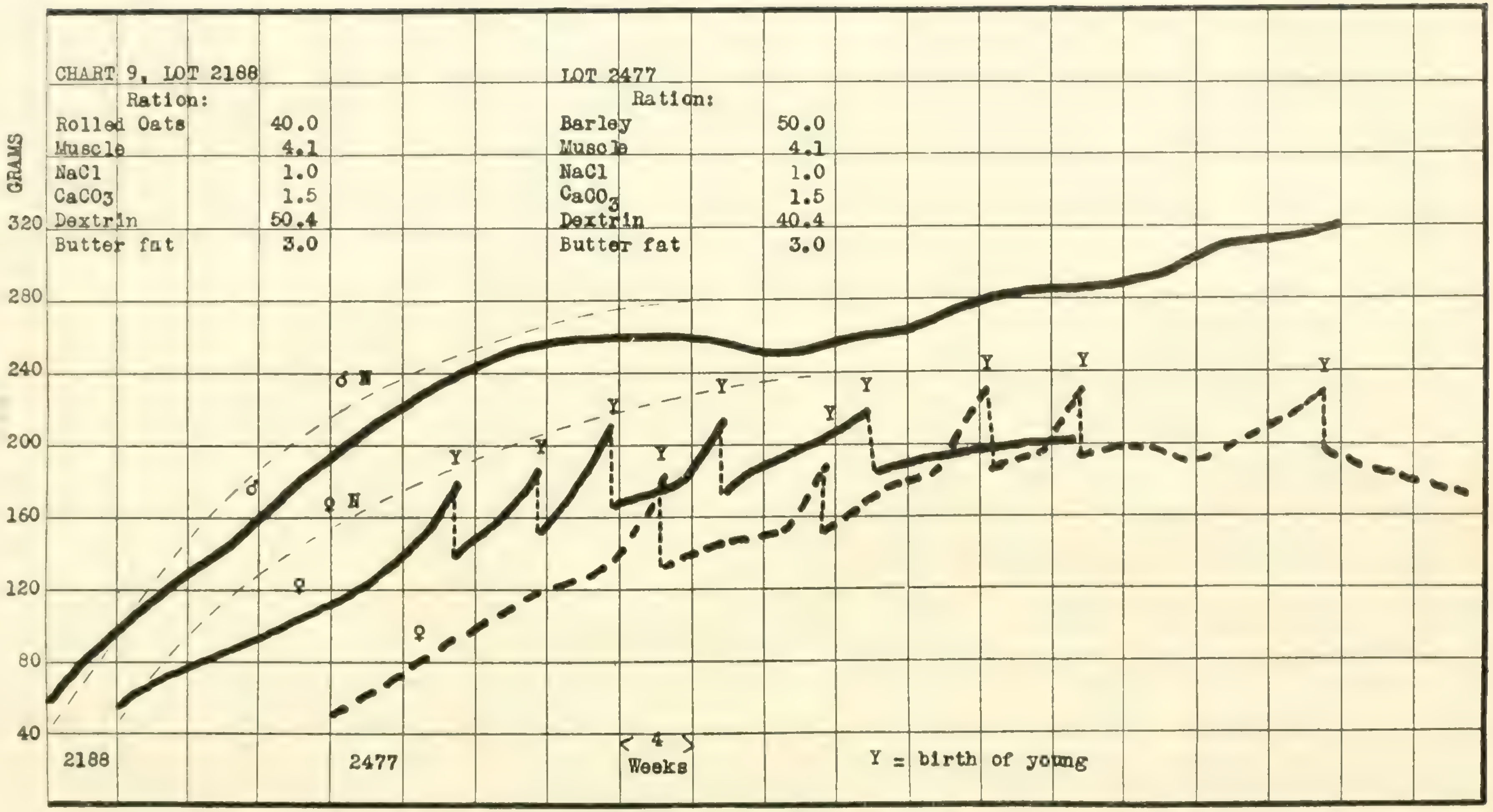


CHART 9, LOT 2188

Ration:	
Rolled Oats	40.0
Muscle	4.1
NaCl	1.0
$CaCO_3$	1.5
Dextrin	50.4
Butter fat	3.0

LOT 2477

Ration:	
Barley	50.0
Muscle	4.1
NaCl	1.0
$CaCO_3$	1.5
Dextrin	40.4
Butter fat	3.0

and returned to the family cage they were killed by the older animals.

Chart 10.—Lot 2187 was fed 6 per cent of maize protein and 3 per cent of muscle protein. Inorganic and fat-soluble A additions were made to correct the deficiencies in the maize and muscle mixture. The growth curves of this lot were distinctly below the optimum and inferior to the group fed wheat and muscle proteins. At 16 to 17 months they were very old looking and their offspring all presented an inferior appearance.

Two females had collectively thirty-three young (six litters) and weaned thirteen. Two more litters were born but were killed by the mothers immediately after birth and the number was not ascertained. The nursing period in these animals was greatly prolonged because of the stunted condition of the young. Two females of the second generation were confined to this diet. They grew very poorly and never had any young.

Lot 2183 was fed 9 per cent of protein derived from peas (6 per cent) and muscle (3 per cent). The growth of the rats restricted to this diet was not uniformily good, some reaching the full adult size in the usual period for well fed rats, others remaining permanently undersized. The curves of growth were far superior to any we have ever seen on 9 per cent of pea protein alone (Chart 1, Lot 2376). They were rough coated, old looking, and irritable at 12 months of age.

Two females had each a litter of young (seven young collectively) and destroyed them soon after birth.

Lot 2185 was fed 9 per cent of protein derived from soy beans (6 per cent) and muscle (3 per cent). The growth records were distinctly superior to those we have secured on 9 per cent of soy beans alone (Chart 1, Lot 2510). Their span of life was short. They were very old looking at 14 months.

There was but one female in this group. She had two litters (eight young) but destroyed them soon after they were born.

Chart 11.—Lot 2478 was fed a diet, the protein of which was furnished by barley and liver. It was comparable to those previously discussed in which the protein was derived from cereal or legumes, supplemented with kidney or steak, respectively. The growth and reproduction records of these animals show clearly that liver is superior to muscle as a supplement to barley (compare

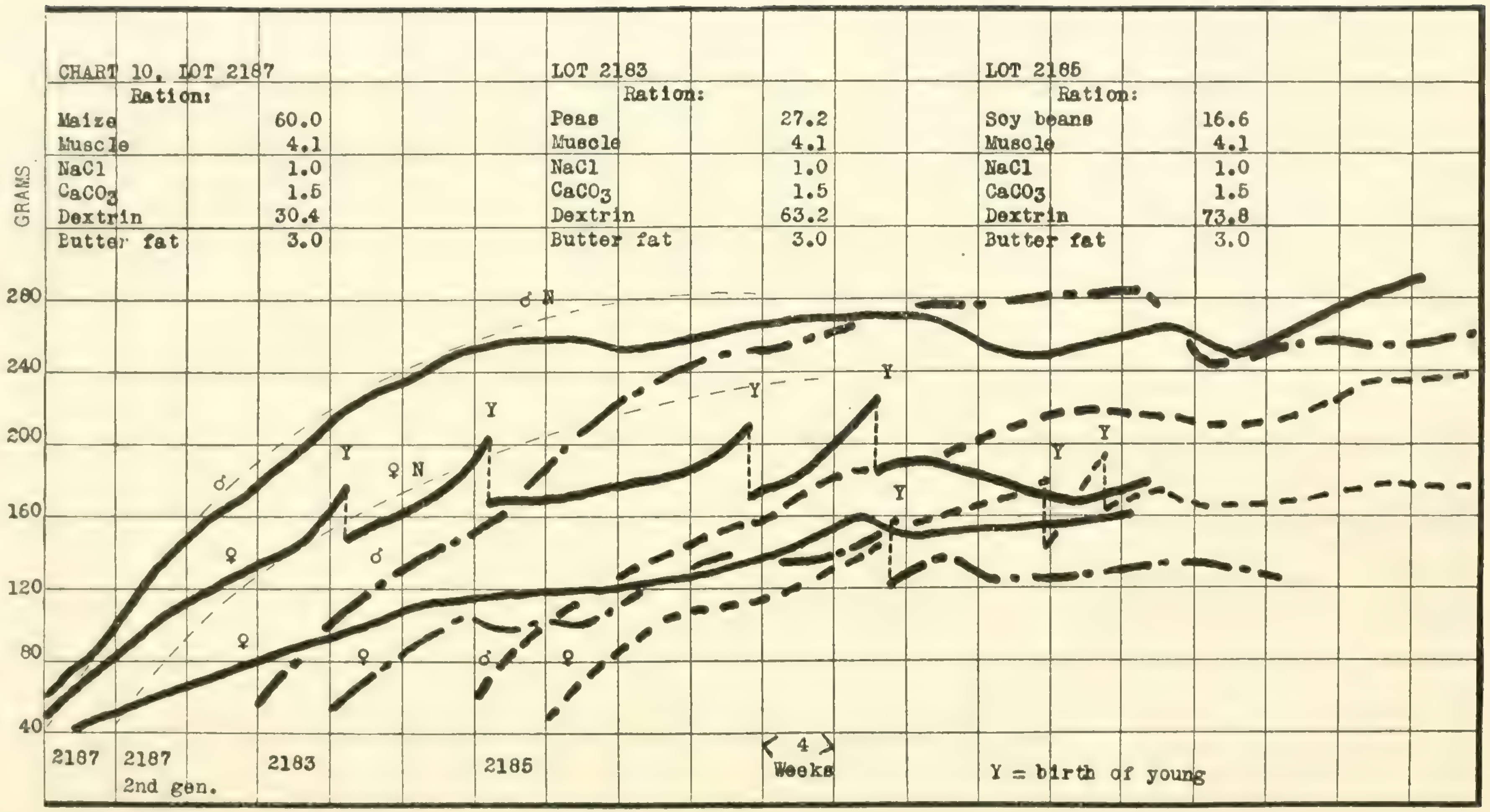


CHART 10. LOT 2187
Ration:
Maize 60.0
Muscle 4.1
NaCl 1.0
CaCO3 1.5
Dextrin 30.4
Butter fat 3.0
LOT 2183
Ration:
Peas 27.2
Muscle 4.1
NaCl 1.0
CaCO3 1.5
Dextrin 63.2
Butter fat 3.0
LOT 2185
Ration:
Soy beans 16.6
Muscle 4.1
NaCl 1.0
CaCO3 1.5
Dextrin 73.8
Butter fat 3.0
GRAMS
280
240
200
160
120
80
40
♂ N
♀ N
♂
♀
Y
2187
2187
2nd gen.
2183
2185
4
Weeks
Y = birth of young

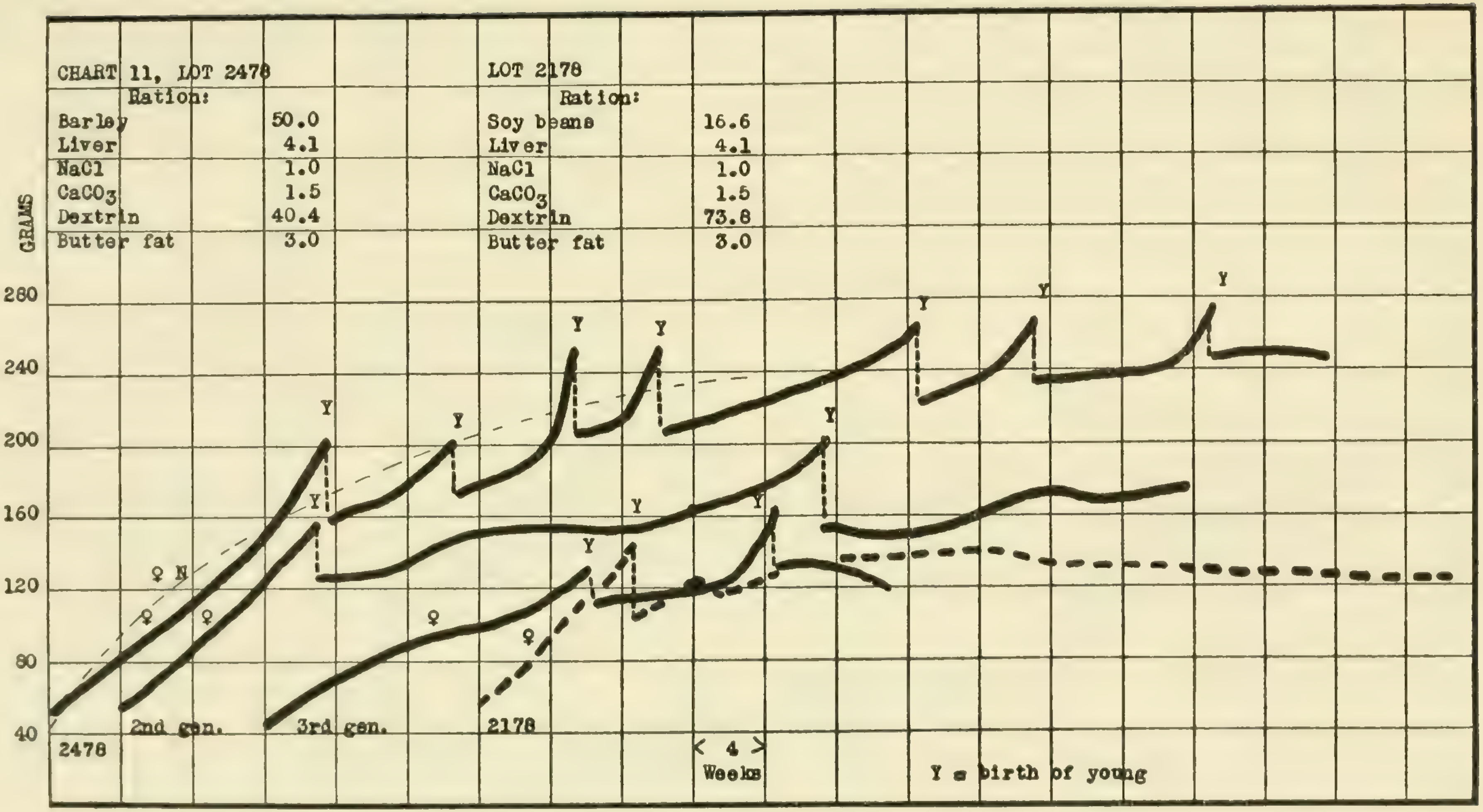


CHART 11, LOT 2478
Ration:
Barley 50.0
Liver 4.1
NaCl 1.0
CaCO3 1.5
Dextrin 40.4
Butter fat 3.0
LOT 2178
Ration:
Soy beans 16.6
Liver 4.1
NaCl 1.0
CaCO3 1.5
Dextrin 73.8
Butter fat 3.0
GRAMS
280
240
200
160
120
80
40
Y
♀
N
2478
2nd gen.
3rd gen.
2178
< 4 >
Weeks
Y = birth of young

Lot 2477, Chart 9 with Lot 2478, Chart 11). These animals were in good condition at 16 months of age, although their coats were beginning to be rough.

Three females were restricted to the diet. One whose growth was poor was sterile. The other two had collectively fifty-four young (ten litters) and weaned twenty-two. Four second generation females were kept on the diet. Two of these failed to grow in a normal manner and remained sterile. The other two had twenty-six young (four litters) and weaned twenty-five. One third generation female had nine young (two litters) and weaned them all.

Lot 2178 was fed 9 per cent of protein, two-thirds of which was from soy beans and one-third from liver. The growth curves were distinctly below normal, but very much better than could be secured with 9 per cent of soy bean protein alone (Lot 2510, Chart 1). These animals were undersized and old looking at 14 months, which was somewhat earlier than in the case of Lot 2478 fed barley and liver, and distinctly earlier than the time of appearance of senile characteristics in rats fed wheat and liver (Lot 2179, Chart 15).

Two females had collectively eighteen young (three litters) and destroyed them soon after birth.

Chart 12.—Lot 2180 was fed maize and liver proteins as 9 per cent of the food mixture. As in the other diets described in this paper the cereal furnished 6 per cent and the glandular tissue 3 per cent. These growth curves were superior to what could be secured on 9 per cent of maize protein alone, but not so good as were observed in rats fed oats and liver (Lot 2181, Chart 13) or wheat and liver (Lot 2179, Chart 15). These animals were very old looking at 16 months and the young were always undersized and inferior in appearance.

Two females had collectively fifty young (seven litters) and weaned but eight. The cause of the infant mortality in this family was mainly due to inadequate nutrition during the nursing period. This was greatly prolonged because of the stunted condition of the young. Those restricted to the diet after weaning did not thrive. Even in litters which were steadily reduced by the death of individuals at intervals of several days, the survivors did not grow better because of the lessened competition for the mother's milk supply.

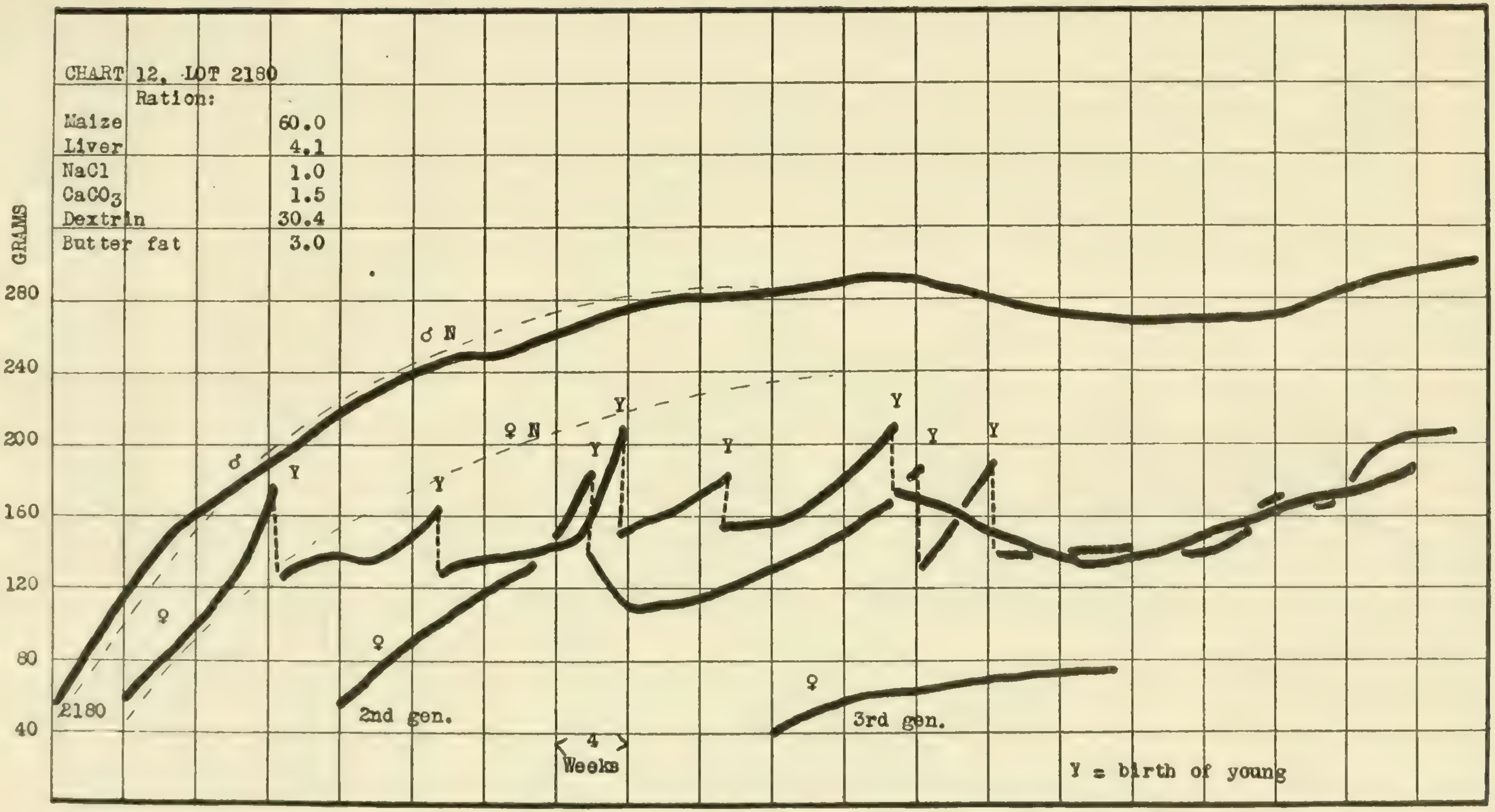


CHART 12. LOT 2180
Ration:
Maize 60.0
Liver 4.1
NaCl 1.0
CaCO3 1.5
Dextrin 30.4
Butter fat 3.0
GRAMS
280
240
200
160
120
80
40
♂ N
♀ N
♂
♀
Y
2180
2nd gen.
3rd gen.
4 Weeks
Y = birth of young

Two second generation females grew up on the diet and had collectively twenty-seven young (four litters) and weaned fourteen of them. One third generation female was restricted to the diet but never grew beyond about 75 gm.

A record of infant mortality such as that of this group we know to be the result of poor quality or lack of sufficient quantity of milk secreted by the mother. It seems justifiable to conclude that it was quality rather than the amount of milk which was the determining factor, since the nursing periods were in most cases so prolonged beyond the normal period of 25 days. If the milk flow had been low it would hardly have persisted over so long a period and have enabled a few young to at last reach a state of independence. The young in the second generation were distinctly better once they became able to eat the diet on which the mother was producing milk, than they were while still so immature as to be confined to their mothers' milk as their sole food.

Chart 13.—Lot 2181 was restricted to a diet of rolled oats and liver, the plane of protein intake being 9 per cent of the food mixture. The oats supplied 6 per cent and the liver 3 per cent of the total protein. The inorganic and fat-soluble A deficiencies were made good by suitable additions of salts and butter fat. The growth curves of the group were normal and fertility essentially so, but the mortality of the young was high. The rats in this group began to look old at 14 months and deteriorated rapidly thereafter. The growth curves of this group were far superior to those of Lot 2188 (Chart 9) which grew up on a similar diet with beef muscle in place of liver.

Two females in this group had collectively sixty-eight young (ten litters) and weaned forty. Two second generation females had forty-three young (ten litters) and weaned fourteen. One third generation female had a litter of seven when she had been 6 months on the diet after weaning. She weaned six of these.

All the animals of this group were killed at about 18 months of age, and while they presented shabby exteriors their organs were found at autopsy to be in good condition and there were no lung infections visible.

Chart 14.—Lot 2182 was fed 9 per cent of protein, 6 per cent being furnished by rye and 3 per cent by liver. The growth curves were excellent and the fertility was high. This shows that this

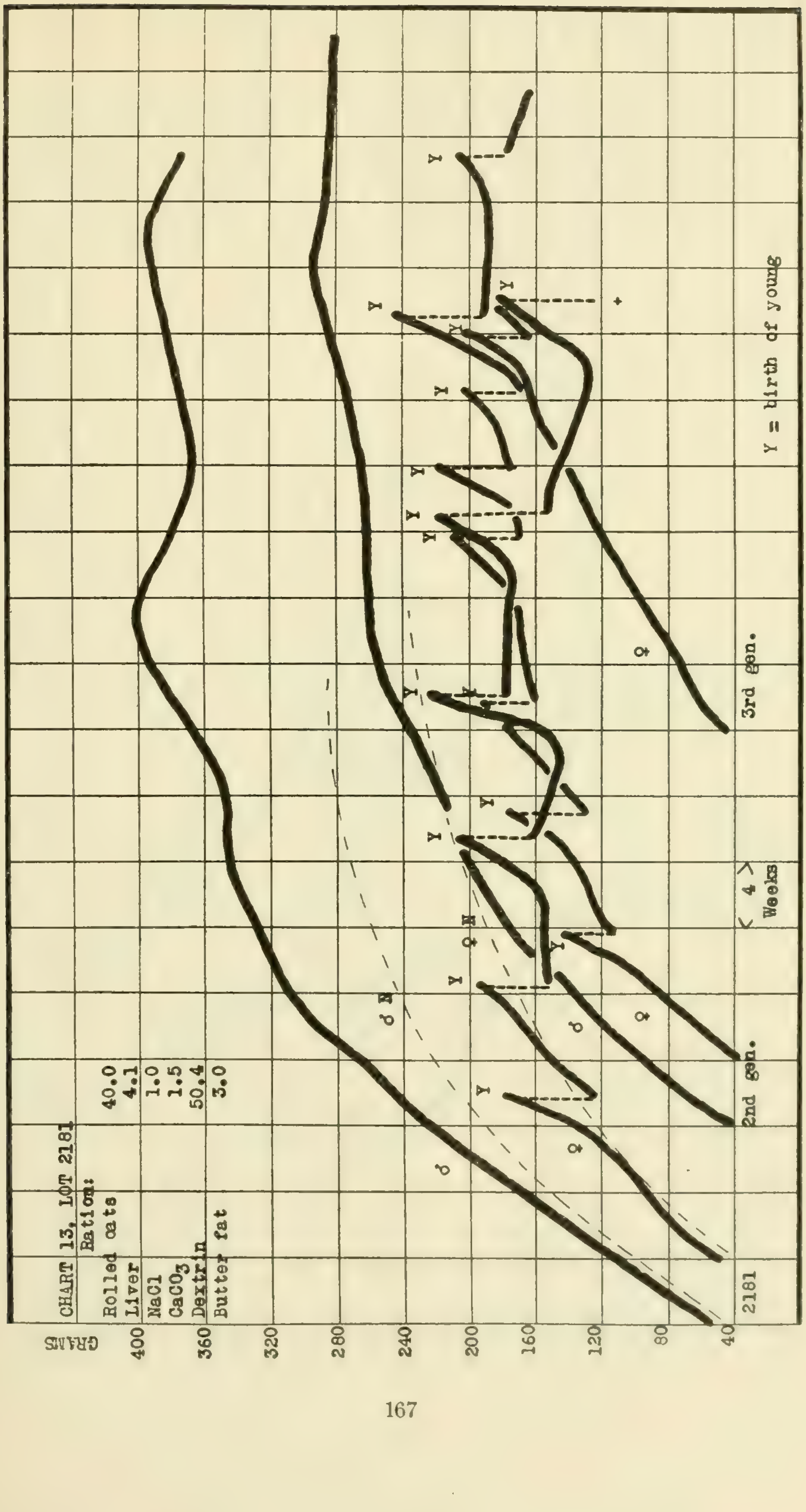


CHART 13, LOT 2181
Ration:
Rolled oats 40.0
Liver 4.1
NaCl 1.0
$CaCO_3$ 1.5
Dextrin 50.4
Butter fat 3.0
GRAMS
400
360
320
280
240
200
160
120
80
40
2181
2nd gen.
3rd gen.
< 4 >
Weeks
Y = birth of young
♂
♀
♂ N
♀ N
Y

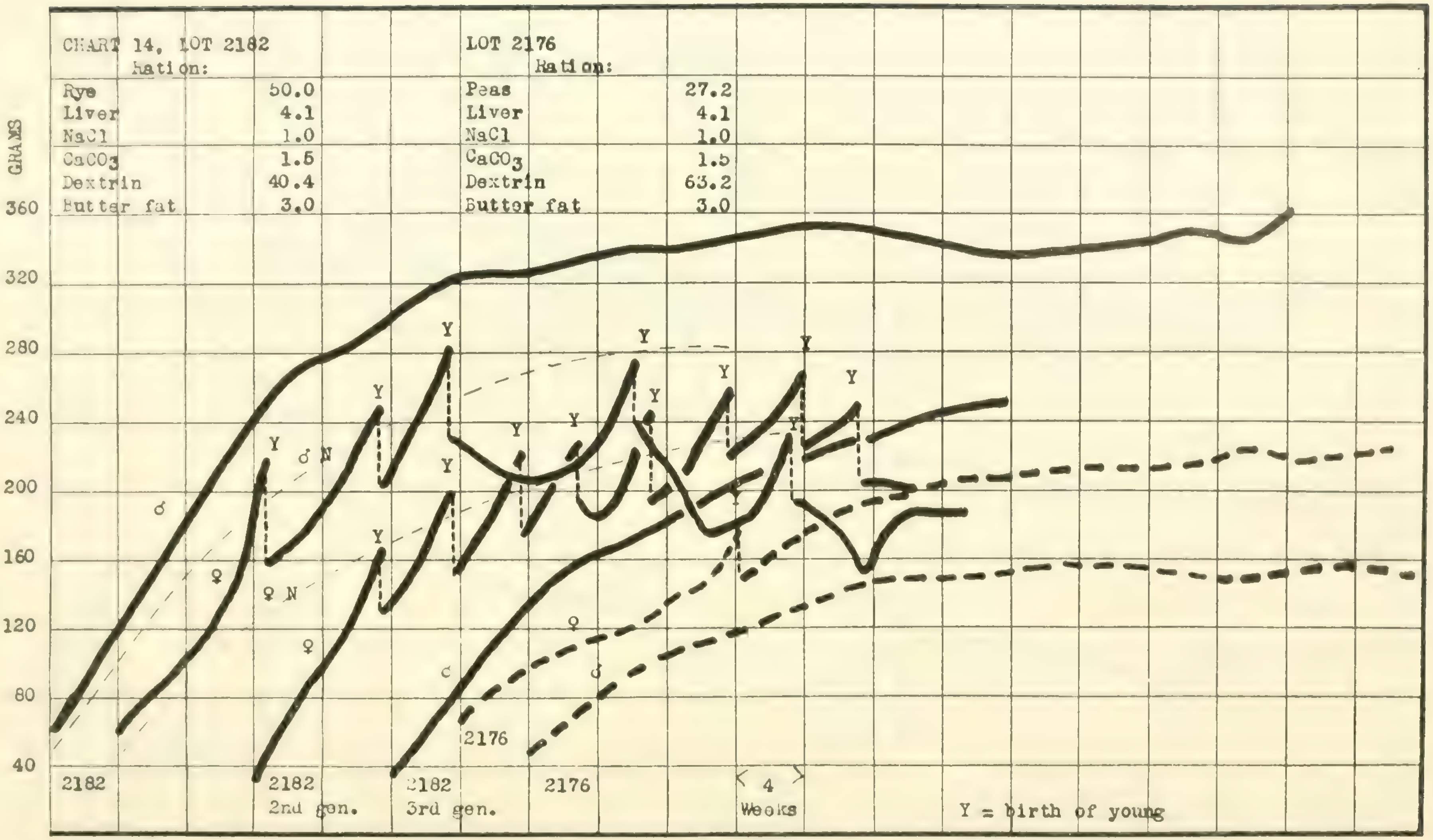


CHART 14, LOT 2182
Ration:

Rye	50.0
Liver	4.1
NaCl	1.0
$CaCO_3$	1.5
Dextrin	40.4
Butter fat	3.0

LOT 2176
Ration:

Peas	27.2
Liver	4.1
NaCl	1.0
$CaCO_3$	1.5
Dextrin	63.2
Butter fat	3.0

combination of rye and liver proteins is of excellent quality, otherwise 9 per cent of protein would not have sufficed to promote growth so satisfactorily. The infant mortality of this group was, however, very high. This was due to the inadequacy of this amount of protein, even of excellent quality for growth, for the elaboration of milk of suitable quality for the nutrition of the young.

Two females had collectively sixty-one young (nine litters) and weaned thirty-four of them. Three second generation females had fifty young (ten litters) and weaned only eight of them. One of these three had a litter of three and thereafter went 12½ months before having a second litter. One rat (a male) of the third generation was restricted to the diet and grew to a size nearly normal to the adult.

The rats in this group were first observed to show the coat changes characteristic of incipient retrogression toward senility at about 15 to 16 months.

Lot 2176 was fed a 9 per cent diet derived from peas (6 per cent) and liver (3 per cent). The growth curves were poor as compared with any secured with combinations of cereals and liver in comparable amounts. They were undersized and their fertility was low. They assumed the characteristic coat which we have taken as an index to the transition from the youthful to the senile condition at about 13 months.

Three females were restricted to this diet. One had a litter of five young but destroyed them soon after birth. The other two remained sterile.

Chart 15.—Lot 2179 was fed 9 per cent of protein, 6 per cent being derived from wheat and 3 per cent from liver. Their growth curves were normal but they were never in so good a condition as those fed either oats or rye with liver (Lot 2181, Chart 13 and Lot 2182, Chart 14). Their fertility was very high but the mortality of the young was great. They began to look old at 18 months of age.

Two females had collectively 127 young (eighteen litters) and weaned forty-two. Most of these young were destroyed by the mothers soon after birth. The mothers were apparently in good condition and the destruction of the young can be accounted for only on the basis of a peculiar psychological reaction of the mother owing to the nature of the faulty diet.

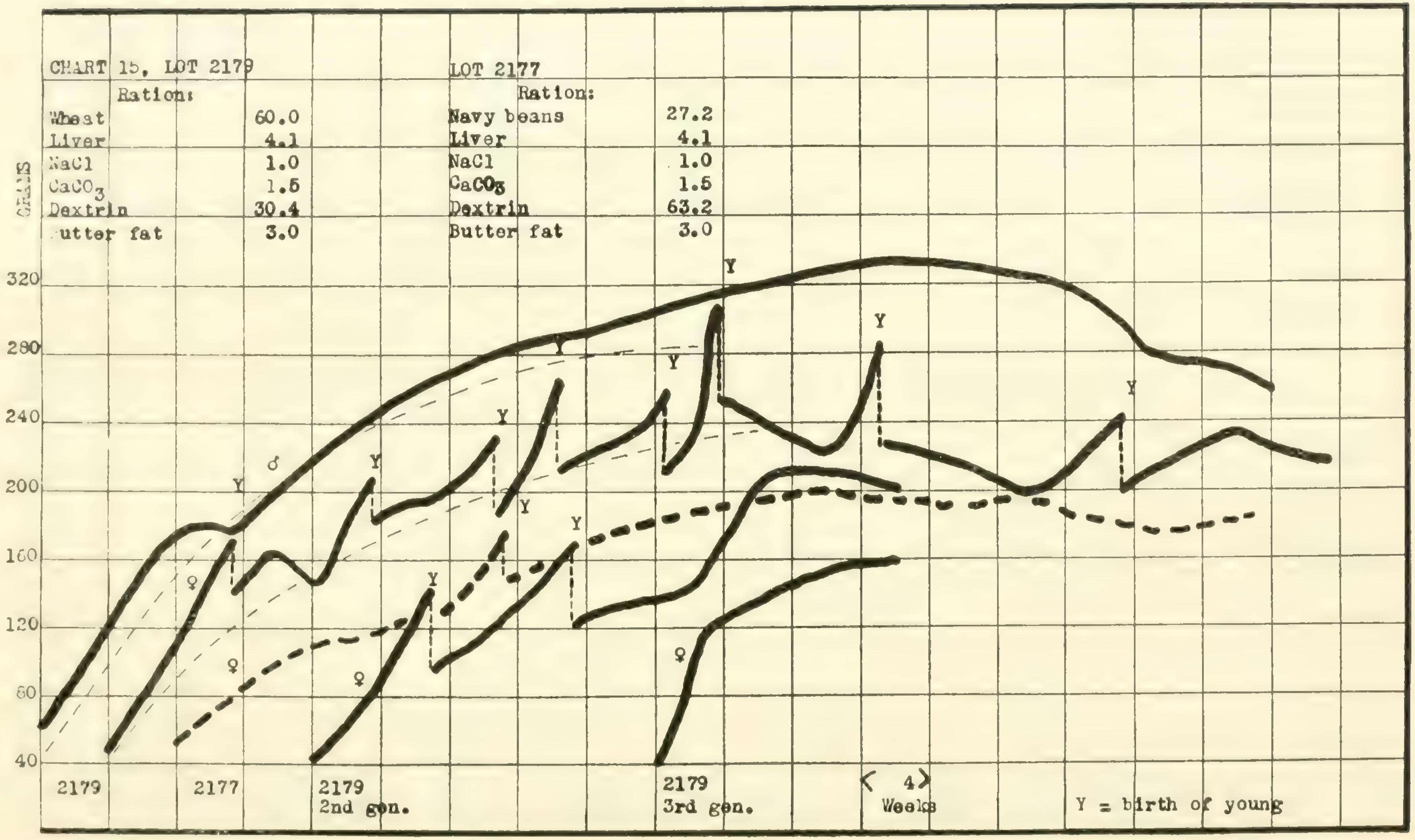


CHART 15, LOT 2179
Ration:
Wheat 60.0
Liver 4.1
NaCl 1.0
CaCO3 1.5
Dextrin 30.4
Butter fat 3.0
LOT 2177
Ration:
Navy beans 27.2
Liver 4.1
NaCl 1.0
CaCO3 1.5
Dextrin 63.2
Butter fat 3.0
GRAMS
320
280
240
200
160
120
80
40
Y
♂
♀
2179
2177
2179
2nd gen.
2179
3rd gen.
< 4 >
Weeks
Y = birth of young

Three second generation females had collectively twenty-three young (four litters) and weaned only one. This sole survivor was kept on the diet for 3½ months but was inferior in development and was discarded.

Diets of this type in which the protein intake was about 18 per cent, and in which 11 per cent was furnished by cereals and legume seeds and 7 per cent by liver, kidney, or muscle, have proved excellent for promoting fertility and rearing of young with very low mortality. This proves conclusively that in the series of experiments reported in this paper the limiting factor in growth, fertility, and success in rearing young was protein.

Lot 2177 was fed navy beans to furnish 6 per cent of protein and liver to furnish 3 per cent. The growth curves were inferior to any observed on combinations of a cereal and liver, but comparable to those of rats fed peas and liver in comparable amounts (Lot 2176, Chart 14). Growth was, however, far superior to any secured with 9 per cent of navy bean protein alone (Lot 2368, Chart 1).

These animals appeared *very old* at 15 months. There were three females in the group. One had a litter of two which she destroyed soon after birth. The other two remained sterile.

Chart 16.—Lot 2392 was fed a diet containing but 4 per cent of protein derived from potato (1 per cent) and liver (3 per cent). A very small amount of nitrogen in this and the following rations was added in the alcoholic extract of wheat germ. The rats were unable to grow on this diet, all factors in which, other than protein, were of fairly satisfactory quality. In Period 2 purified protein (casein) was added. This modification of the diet was followed by a sharp response to growth.

Lot 2394 was fed a diet identical with that of Lot 2392 except that muscle tissue (round steak) replaced the liver. The animals grew very slowly on this food mixture containing but 4 per cent of protein. In Period 2 the protein moiety of the diet was enhanced by the addition of casein. This led to growth at a rapid rate.

Lot 2393 was fed a diet identical with those of Lots 2392 and 2394 except that kidney replaced the liver and muscle, respectively. This diet also contained 4 per cent of protein. Growth at a slow rate was possible on this diet. In Period 2 casein was added and this led to a stimulation of growth. It will be seen from the curves

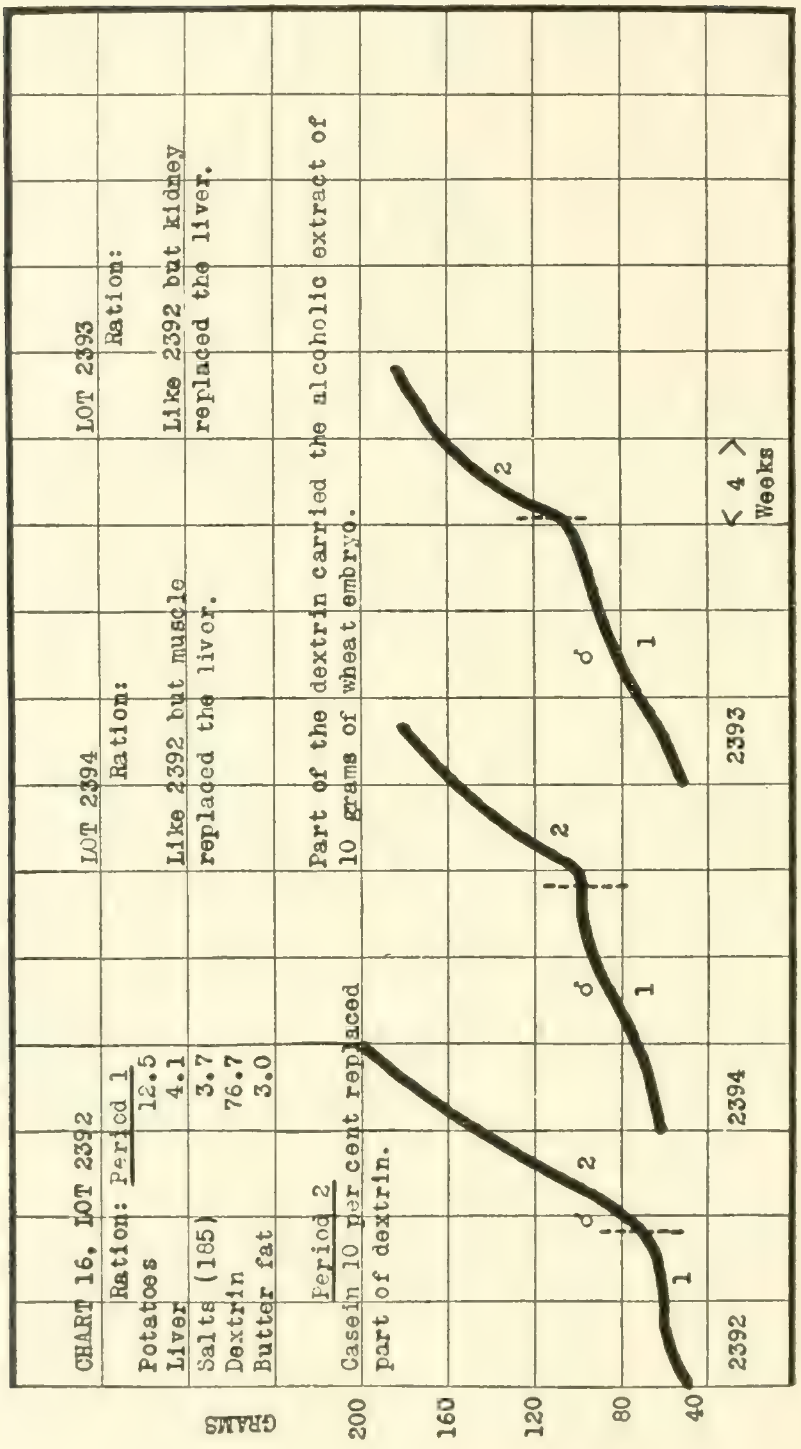


CHART 16, LOT 2392
Ration: Period 1
Potatoes 12.5
Liver 4.1
Salts (185) 3.7
Dextrin 76.7
Butter fat 3.0
Period 2
Casein 10 per cent replaced part of dextrin.
LOT 2394
Ration:
Like 2392 but muscle replaced the liver.
Part of the dextrin carried the alcoholic extract of 10 grams of wheat embryo.
LOT 2393
Ration:
Like 2392 but kidney replaced the liver.
GRAMS
200
160
120
80
40
1
♂
2
2392
2394
2393
< 4 >
Weeks

presented in the chart, each of which is representative of a group of four animals, that kidney protein supplements the nitrogen of the potato better than either liver or muscle proteins. These curves are included in this series to illustrate the fact that certain combinations of proteins are of sufficiently good quality to enable young rats to grow nearly as well on diets containing but 4 per cent of protein (potato and kidney) as they do on rations of similar value with respect to non-protein factors in which the sole source of nitrogen is 9 per cent of legume proteins (pea, navy bean, and soy bean). Compare Chart 1, Lots 2376, 2368, and 2510 with Chart 16.

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SUPPLEMENTARY PROTEIN VALUES IN FOODS.

III. THE SUPPLEMENTARY DIETARY RELATIONS BETWEEN THE PROTEINS OF THE CEREAL GRAINS AND THE POTATO.

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It has become well established that the diet of man or animals may, when defective in sufficient degree, produce at least two distinct syndromes, scurvy and beri-beri. Experimental studies on animals leave no room for doubt that a third specific deficiency disease, xerophthalmia or keratomalacia, is produced by lack of a definite organic factor essential for normal nutrition. There is much reason to suspect that certain eye conditions popularly referred to as night-blindness, and in the literature as hemeralopia may be, in part at least, due to lack of sufficient fat-soluble A. The widespread infections of the eyes seen in Egypt and elsewhere, may have their origin in lowered vitality of the eye structures due to lack of the dietary factor, fat-soluble A, which favors the invasion of the eyes by microorganisms. The evidence seems sufficient to warrant the conclusion that there are three deficiency diseases which occur in man as well as in animals.

It is natural that the first step toward an appreciation of the relation of the diet to health in man should have been the result of casual observation on the extreme conditions, scurvy and beri-beri. The rarity of reference to the relation of faulty diet to the general health of peoples in the writings of those who are now discussing health problems, shows that it is not at present accepted by many that there is any important relation of the food supply to well being, except in extreme cases of one-sided diet in which the defects are sufficiently grave to induce the development of a "deficiency" disease. The reason for this is easy to understand. Our standards of what constitutes wellness and illness, as well as of what constitutes normal physical development, are relatively low. People are generally considered well until they admit that they are ill, or until they look so unwell as to attract attention. The difference between the average physical development of men and women, as contrasted with the best specimens in the community, is very great, yet we are accustomed to regard as normal many persons who are but poorly constituted physically.

Malnutrition is painless, and leads to susceptibility to other agencies which may induce discomfort. The sequence of events is not ordinarily appreciated, and the cause, therefore, escapes notice. Low vitality, low resistance, and inefficiency, and a tendency to cumulative fatigue, are what we should expect in man to result from adherence to a faulty diet when the faults are of a lower order than would be necessary to bring about an attack of a "deficiency" disease. Although a few leaders in the new movement for the betterment of the health condition of children are urging the importance of properly selected food, the idea is new and not generally appreciated. There is much reason to believe that one of the most important contributing factors in industrial fatigue is the low recuperative power that results from an unsatisfactorily selected food supply. The data regarding the animals described in this paper, all of which were subjected to diets which were faulty to a certain extent, afford striking evidence that such types of diets interfere with the completion of the life history in a normal manner. This is true, notwithstanding the fact that in some instances the faults were by no means marked. The extent to which the animals were injured, and the manner in which they were affected, afford food for serious reflection concerning the similar experience of man and its probable causes.

The results of our experimental studies on the rat have led us to an appreciation of the short-sightedness of the view that a diet is satisfactory if it is not sufficiently poor to cause the development of a "deficiency" disease. Although the importance of a diet containing an abundance of milk and eggs has become generally accepted to have a distinct therapeutic value in the treatment of tuberculosis, it has not been widely appreciated that a similar diet should be very effective as a preventive of infection or of the flaring up of the disease.

We have given a great deal of attention to the problem of demonstrating the effects of relatively slight defects in the diet on the general health of the rat, and on its capacity to reproduce and rear young, and to remain vigorous to an advanced age. We have united the study of these problems with the study of the extent to which the proteins of certain foods supplement those of others. It has been easily possible to develop these lines together, and indeed it was not possible to pursue the one phase of the work independently of the other, for in such systematic studies one meets with the nice gradations in quality of proteins which afford the opportunity to make refined observations on the physiological well being of the animals as affected by the variations in the diet.

We deem it a matter of great importance to discover in its incipiency when an animal is brought into a state of nutritive instability. Under such conditions it is unable to support a burden of any appreciable magnitude, and it matters little whether the burden be that of producing milk, or bearing young, or of withstanding fatigue. It is such shades of difference in quality which occur in human experience in numerous cases. The type of experiment which we have come to employ, in which the performance of reproduction and nursing of young comes under observation, brings to light this "twilight zone" of nutrition in which the vital powers of the body are below the optimum.

It is desirable at this time to discuss the changes in appearance of the rat, and in its behavior as it grows old, in order to make possible an appreciation of the manner in which we have managed our experiments.

The well fed rat exhibits up to about 18 months of age the appearance of full vigor as shown by plumpness of the muscles; bright, prominent eyes; clean, smooth, pink skin, easily observable on the feet and ears. The tail appears clean and free from scales. The coat appears to consist of hairs of approximately uniform length, and is glossy and well kept. Such animals are not apprehensive and permit themselves to be handled without becoming annoyed. They do not attempt to escape when handled with care.

At a certain point in their life history there is a characteristic change in the appearance of the coat. This consists of the prominence of certain hairs which are scattered over the entire body, and which look coarse and are distinctly longer than the under coat.

Physiologically youthful rats, irrespective of age, give attention to their coats. When the latter differentiates into hairs of two sorts the animals become neglectful of their toilets and look less clean and attractive than formerly. The skin on the ears, feet, and tail generally loses its waxy and healthy surface and appears dry, harsh, and often scaly.

With this change in appearance there is usually seen a change in the habits of the animals. They tend to sit and sleep with the head tucked under the body or with the face downward. Healthy youthful rats sleep lying stretched out on their ventral surface or partly turned on one side, with the front legs extended and with the head resting on the paws. When the cage of youthful rats is opened they are awakened if asleep, and show an interest in their observer, but do not show signs of fear. Animals which are developing senile characters are generally dozing and their attention is not usually attracted by careful opening of the cage. When touched they spring up in surprise and show irritation at being molested. As time passes this behavior becomes more marked. The irritability, somnolence, dirtiness, roughness of the coat, and scantiness of hair become progressively more noticeable. To some extent, according to the nature of the fault in the diet, specific changes appear, such as attenuation of form and baldness over limited areas, but these latter are more characteristic of animals fed certain foods than they are of diets from different sources having like specific dietary deficiencies. A further example of this peculiar effect of diet due to some obscure causes is seen in the frequent occurrence of very fine, short, glossy hair, suggestive of a mole in rats fed largely on maize or kafir corn.[1]

Another type of observation which has proved to be of value as an index to the quality of the diet which enables one to make fine shades of distinction in the value of the protein factor in a series of experiments which are properly planned, is the success of the females in rearing their young. This is a more sensitive index than is fertility, although in general low fertility and high infant mortality tend to run parellel. The fertility may, however, be high, and but a small number of young be reared to the weaning age. This mortality is, we believe, generally due to poor quality of the milk secreted by the mothers rather than lack of milk production. It is not always possible to get satisfactory information on this point, but since the stomach when filled with milk can be readily seen in a young rat during the first few days when the animal is properly illuminated, we have been able to observe the amount of milk produced in a sufficient number of cases to lead us to believe that in general the failure of the young to grow is due to deficiencies in the composition of the milk rather than to lack of sufficient amount.

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[1] McCollum, E. V., and Simmonds, N., Unpublished data.

There is a marked tendency among female rats fed such diets as are described in this paper to destroy their new-born young. If for any reason they become scattered about the cage the mothers will not be solicitous about returning them. This abnormal psychological reaction of the mothers toward their young represents a new and hitherto unstudied effect of faulty nutrition. It serves to emphasize the deep seated relation of the character of the diet to the mental reactions generally, for if the maternal instinct is so perverted, those attributes of the nervous system which are less deeply ingrained should be influenced in even greater degree.

In contrast to the behavior of the mothers on experimental diets, details of which are given in the legends to the charts, it is of interest to contrast the behavior of the mothers in our breeding stock. It is our custom to isolate the females when it is easily observable that they are pregnant. This cannot be determined with certainty until about the end of the second week after fertilization. For some years it has been our custom to isolate two or three females in our breeding stock in a single cage. They each prepare a nest for the reception of their young, but when the cage is cleaned within a few days, and fresh bedding replaces the old, the young are mixed and returned to the cage together. From this time on the mothers do not separate their own young, but allow them to remain together in one nest, which may contain as many as twenty to thirty individuals. They nurse each other's young indiscriminately. A mother will spread herself over the nurslings, and as many as possible attach themselves to her. After a time she will free herself from them and another mother will take her place. They seldom kill a young one and the infant mortality is almost zero. Only very infrequently have we seen a young rat under such circumstances unable to compete successfully with the others for his share of the milk supply.

In experimental animals on diets which are faulty, it appears, as stated above, that the milk flow is ordinarily sufficient to meet the needs of the developing young, but the quality may be poor with respect to some one or more factors, and cause failure of the animals to grow satisfactorily. It appears, however, that some mothers do not produce enough milk, at least after a time. We have occasionally seen a litter grow fairly well for 4 to 10 days, then all die within a few days. The cause of such behavior has in general not been ascertained.

We have given some attention to determining how long a female rat may continue to give milk provided she is kept with young of a suitable size. In one case in which the young were removed and replaced by smaller ones at intervals of a few days, a female rat continued to secrete milk up to 141 days in quantity sufficient to induce some growth in four young about 8 to 10 days old. It is known that these young did not eat of the mother's food supply.[1]

When the fault or faults in a diet are sufficiently marked, growth is interfered with in the young, and the effects soon become apparent in the external appearance of the animals, even in the adult. We have made an effort to determine *how sensitive* the rat is to slight degrees of faulty composition in its diet. This can be done by prolonging the period of observation far beyond the growth period, and including a record of the fertility and success in rearing young and the time of the appearance of the first signs of old age. Since the character of the diet is but one of the factors which influence the well being of an animal, we have maintained the hygienic conditions such as illumination, temperature, ventilation, and opportunity for exercise uniform throughout our entire rat colony. Furthermore, the differences in the condition and vitality of the animals as measured by the tests described are due in all cases to variation in the quality of the diet, which falls within the limits of what may occur in combinations of ordinary natural foods of animal and vegetable origin.

As an example of the objective which we have had in mind in conducting these studies, the comparison of the relative values of the proteins in the several rations may be cited. The value of the proteins in a food or mixture, or the value of a purified protein, has not been studied by methods more sensitive than that of comparing their efficiency for the support of growth. We have learned that much more accurate comparison of quality in proteins can be made by maintaining a series of animals on a diet otherwise comparable, but containing various levels of the same source of protein throughout the growing period and into adult life, so as to discover the plane which just suffices for this result without inducing inferiority in appearance or size. Under these conditions the mother rat should she produce and attempt to suckle a litter would be confronted with an added demand for nutriment by the young. The young at the nursing age are more sensitive to faulty diets than are older animals which have been satisfactorily nourished during the first weeks of life. We have thus set up experimental conditions in which an unusual demand is made upon the mother, and extraordinary sensitiveness is characteristic of the young because of their early age and lack of reserve power.

It is of more than ordinary interest to find, as we have, that a diet may be sufficiently good to enable a group of animals to grow to apparently the normal adult size, and even to support a fair amount of reproduction and moderate success in the nursing of young to a state of independence, and yet be inadequate for the nourishment of a family through several generations. This fact is brought out well in many of our studies in which families during several generations have been restricted to a monotonous diet. If the diet is properly constituted with respect to all its parts the animals tolerate, at least to the fifth generation, such a monotonous food supply without any signs of injury. If, however, there are factors which are even slightly below the optimum, the vitality of the animals falls off in each succeeding generation, and the strain dies out. Sometimes this happens in the second, third, or fourth generation.

An interesting difference in the effect of stunting of young animals is seen, depending on the cause of the interference with growth. When the protein content of the ration is too low, young rats remain approximately normal in form, or at least they remain long and lithe. When the inorganic content of the ration is faulty on the other hand, they develop a short and stocky form. In the former case they respond to a better protein supply and look approximately normal, but when deformed by lack of sufficient calcium they never assume the normal form as the result of correction of the diet.[1]

The discussion of the growth, fertility, infant mortality, and appearance of the first recognizable signs of senility which is presented in the legends to the charts, will make clear the fact that even very slight differences in the quality of the food mixture with respect to protein and fat-soluble A at least, can exercise a marked influence on the vitality of the rat. Animals may be brought to a state by feeding ordinary cereals and potato, supplemented in a fairly satisfactory manner except for the protein factor, in which their nutritive condition is unstable, and where very slight shifts in the character of the food for better or worse, make easily observable differences in their capacity to perform satisfactorily the functions of reproduction, or to retain full vigor to an advanced age.

The experimental work reported in this paper is limited to a study of diets in which the protein was derived from a mixture of a cereal grain and potato. The quality of protein was estimated as accurately as possible by existing methods. In some cases casein was added to the diet in order to bring out the fact that the protein in a certain mixture was actually the limiting factor. The results show that the potato nitrogen serves to some extent to supplement that of the cereal grains, but that it is not so valuable for this purpose as are certain animal tissues such as kidney, liver, or muscle.[2]

The data furnish definite evidence that there is no marked difference in the capacity of the nitrogen compounds of the potato to enhance those of the different cereal grains, as was found to be the case in similar combinations of cereals with animal tissues. The latter are individually more effective with certain grains than with others, whereas the potato is less highly valuable in any combination, and more similar in its relations to each.

These studies lend support to the general proposition that it is not wise to attempt to combine foods which have the same function, such as have the seeds and tubers. Both are storage tissues of plants and have in great measure the same dietary properties. They do not, therefore, serve effectively to make good each other's shortcomings.

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[2] McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1921, xlvii, 139.

SUMMARY.

We have described methods of experimenting which are more sensitive than rate of growth for yielding information concerning slight gradations of quality in foods. These involve fertility, success with the rearing of young, longevity, the preservation of youthful characters, and the stability of the nervous system. By establishing standards for comparison these indexes to well being reveal with great refinement the physiological condition of the animal.

The supplementary values of the nitrogen compounds of the potato for those of the cereal grains and legume seeds have been studied with a view to making refined observations as to the biological values of several combinations. These experiments have brought to light the following facts:

The nitrogenous compounds of the potato tend in some degree to enhance the biological value of proteins of cereals and legume seeds, but not to as great an extent as do the proteins of such animal products as kidney, liver, muscle,[2] or milk.

Marked differences are observed in the extent to which animal tissues enhance the quality of the proteins of individual seeds. No such selective efficiency has been observed in the relation of the amino-acids of the potato to those of cereal or legume seeds. The potato seems about equally effective as a supplement for all which have been studied.

Chart 1.—These records are typical growth curves of rats fed diets in which wheat, rolled oats, maize, peas, and millet seed, respectively, furnish the only source of protein. The amount of each seed was adjusted so as to make the protein content of the diets 9 per cent of the food mixture. In each case the necessary inorganic salts and butter fat were added to make good the well established deficiencies of these seeds.

Wheat proteins are seen to produce the best growth curves and the highest fertility. No young were secured from the animals fed maize, peas, or millet seed. A few young were reared on the wheat and oat diets. Charts 5 and 8 show that the infant mortality is in a great measure due to inadequate protein moiety of these diets. These records serve as the basis of comparison with those contained in the other charts.

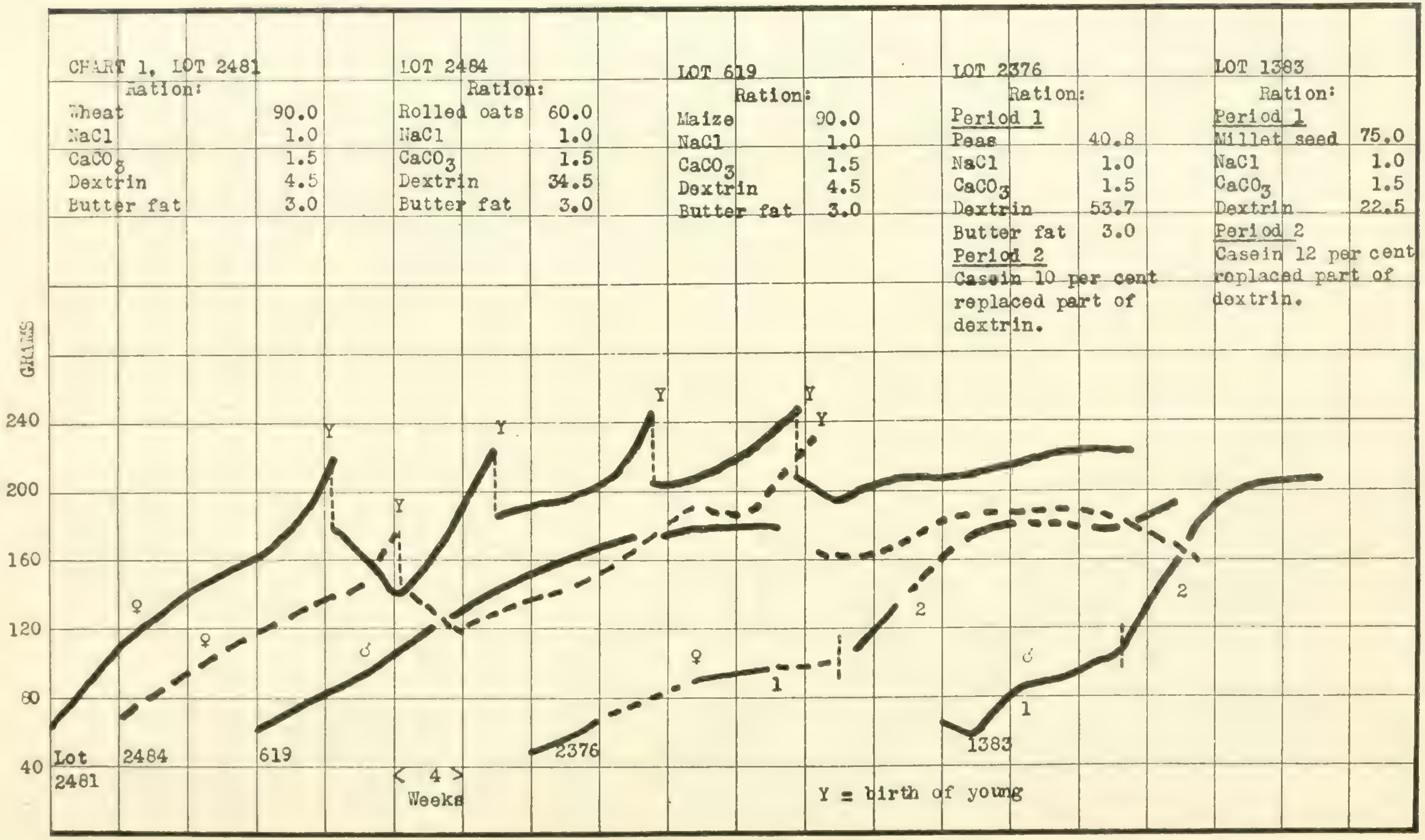

CHART 1, LOT 2481

Ration:	
Wheat	90.0
NaCl	1.0
$CaCO_3$	1.5
Dextrin	4.5
Butter fat	3.0

LOT 2484

Ration:	
Rolled oats	60.0
NaCl	1.0
$CaCO_3$	1.5
Dextrin	34.5
Butter fat	3.0

LOT 619

Ration:	
Maize	90.0
NaCl	1.0
$CaCO_3$	1.5
Dextrin	4.5
Butter fat	3.0

LOT 2376

Ration:

Period 1

Peas	40.8
NaCl	1.0
$CaCO_3$	1.5
Dextrin	53.7
Butter fat	3.0

Period 2

Casein 10 per cent replaced part of dextrin.

LOT 1383

Ration:

Period 1

Millet seed	75.0
NaCl	1.0
$CaCO_3$	1.5
Dextrin	22.5

Period 2

Casein 12 per cent replaced part of dextrin.

In all cases the food mixture was ground together so that all its ingredients were consumed in the proportions shown in the formula, as is our invariable practice.

Chart 2.—These curves illustrate the growth and reproduction of a group of rats fed from weaning time on diets, the protein of which was derived from millet seed and potato. This seed and tuber served as the only source of fat-soluble A.

Lot 1398 contained about 11 per cent of protein (N × 6.25), approximately 9 per cent being derived from millet seed and 2 per cent from potato. The growth records were poor, but distinctly better than was observed in Lot 1383 (Chart 1), in which 9 per cent of protein derived entirely from millet seed was the sole source of nitrogen. No young were secured in the latter group, whereas a few were secured from Lot 1398. This group contained two females. These had together eighteen young (three litters) of which nine were weaned. Two of their daughters were reared on the diet but remained sterile. The young of this group were all undersized for their age and the nursing period had to be prolonged to approximately twice the normal period of 25 days in order to enable the young to survive on the family diet. There is every reason to believe that the high mortality of the young in this group was the result of qualitative inadequacy of the milk secreted by the mothers. All these rats aged early. They were very senile at 8 to 10 months.

Lot 1452 had a diet like that of Lot 1398 except that it contained 10 per cent of casein to supplement the protein. The good effects of this addition are easily seen in the character of the growth curves. Millet seed does not appear to be a very wholesome food grain. The fertility of the females in this group was far below normal and the mortality of the young high. These young were not destroyed soon after birth but only after they had time to show from retarded growth that the quality of the milk of the mother was inferior.

Two females had together twenty-six young (three litters) of which twenty were weaned. Two daughters were confined to this diet. One had one litter of four young which she destroyed. The other had one litter which she destroyed, and died in the delivery of a second litter.

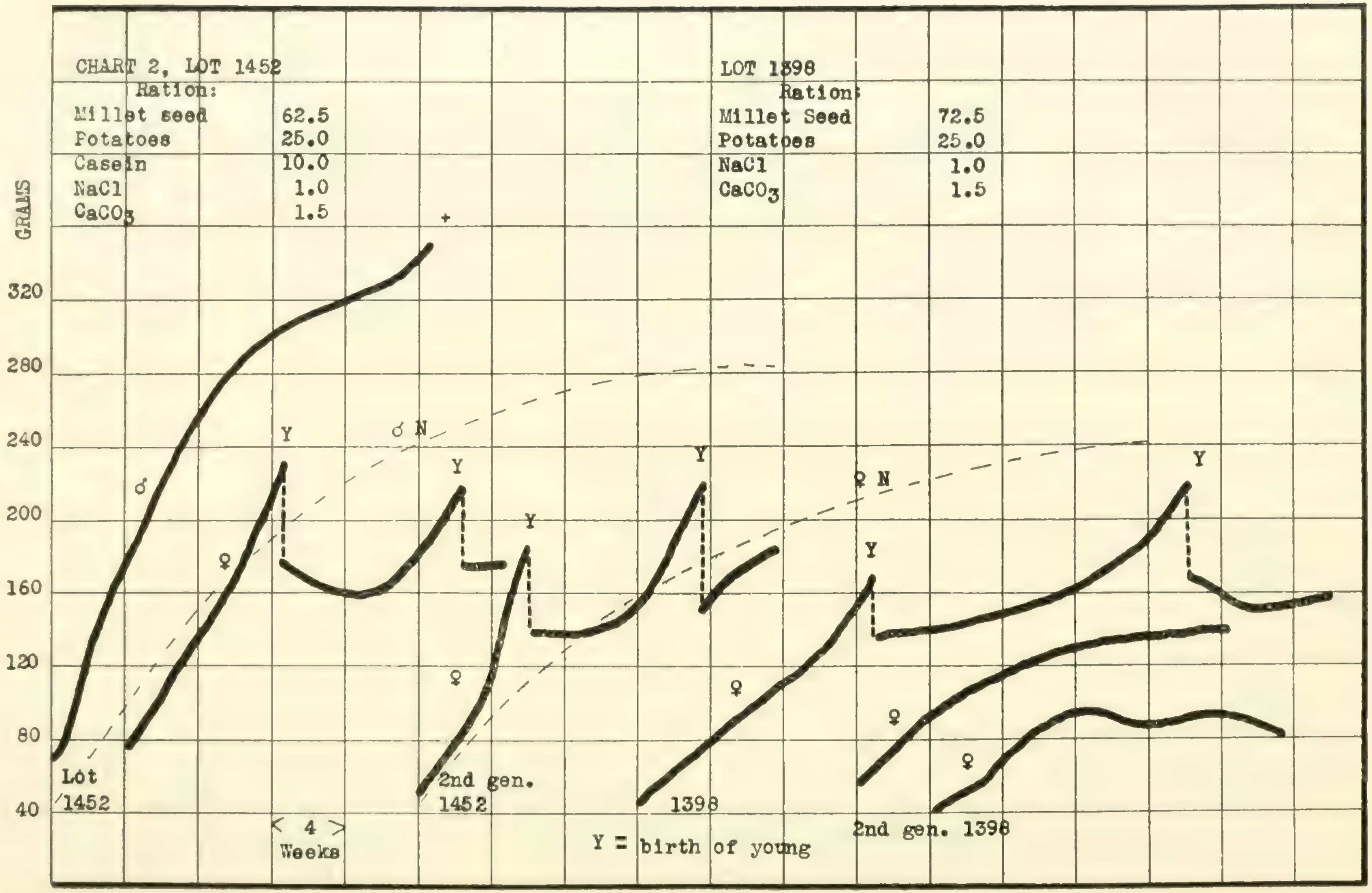

CHART 2, LOT 1452

Chart 3.—The animals in Lots 1407 and 1461 were fed diets similar to those of Lots 1452 and 1398, respectively (Chart 2), except that the former had an added source of fat-soluble A. Since millet seed contains distinctly more of this factor than wheat or oats, or than peas or beans, the effects of adding butter fat to this ration was scarcely noticeable.

Lot 1407 contained three females. They had altogether thirty-four young (five litters) and weaned fourteen of them. Two females of the second generation were confined to this diet. One had a litter of six but they were very puny. Some of these were destroyed when very young. A litter which was nursed 11 days was then only about 60 per cent as heavy as it should have been if the mother's diet had been satisfactory.

Lot 1461 was fed millet seed, potatoes, butter fat, and casein in Period 1. Little growth was possible on this food. In Period 2 sodium chloride and calcium carbonate were added. Growth took place at a rapid rate after this addition was made. There were three females in this group. They had forty-six young (seven litters) of which thirty-one were weaned. One female of the second generation was restricted to the diet and had a litter of eleven young but killed them all, and died herself soon afterward. Other young from the original group on this diet were very inferior. Where these young were not destroyed when very small they remained stunted while nursing. This we interpret as being due to faulty composition of the milk secreted by the mother. It is probable this was the result of some toxic substance passing into the milk from the millet seed.

These records all show that millet seed when fed in liberal amounts causes injury. This we provisionally interpret as due to the presence of some toxic substance in millet seed. That a shortage of fat-soluble A was not responsible for the results observed is shown in Chart 2. Among the farmers of the middle west there is a common belief that liberal feeding of millet causes injury to farm animals. The toxicity of millet seed is still under investigation.

Chart 4.—The records in this chart are those of rats which were fed diets in which wheat and potatoes furnished the sole source of fat-soluble A. The protein content in the diet of Lot 1399 was approximately 9 per cent. That this amount of protein from these

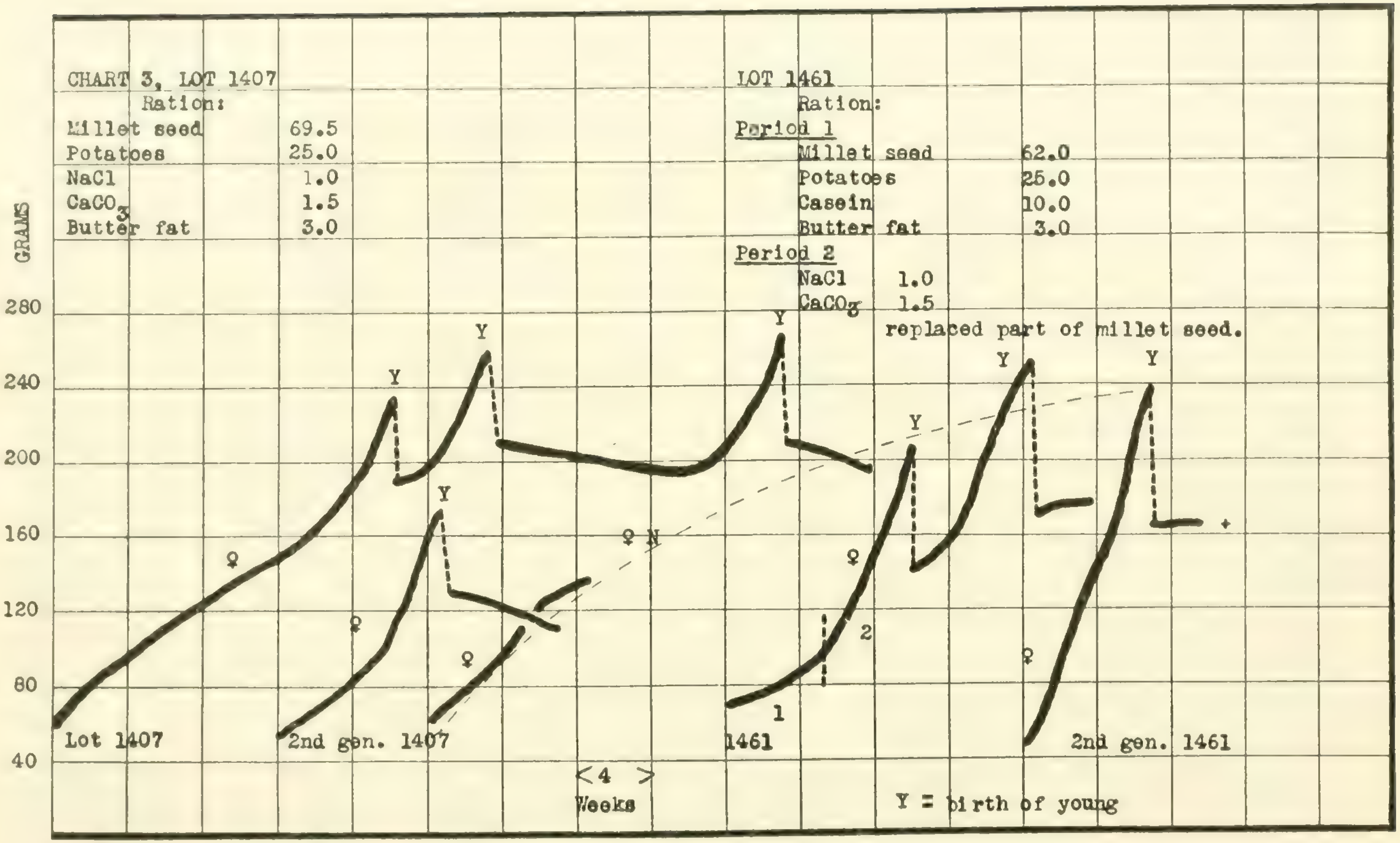
CHART 3, LOT 1407
Ration:
Millet seed 69.5
Potatoes 25.0
NaCl 1.0
CaCO3 1.5
Butter fat 3.0
LOT 1461
Ration:
Period 1
Millet seed 62.0
Potatoes 25.0
Casein 10.0
Butter fat 3.0
Period 2
NaCl 1.0
CaCO3 1.5
replaced part of millet seed.
GRAMS
280
240
200
160
120
80
40
Y
Y
Y
Y
Y
Y
Y
♀
♀
♀
♀ N
♀
♀
1
2
+
Lot 1407
2nd gen. 1407
1461
2nd gen. 1461
<4 >
Weeks
Y = birth of young

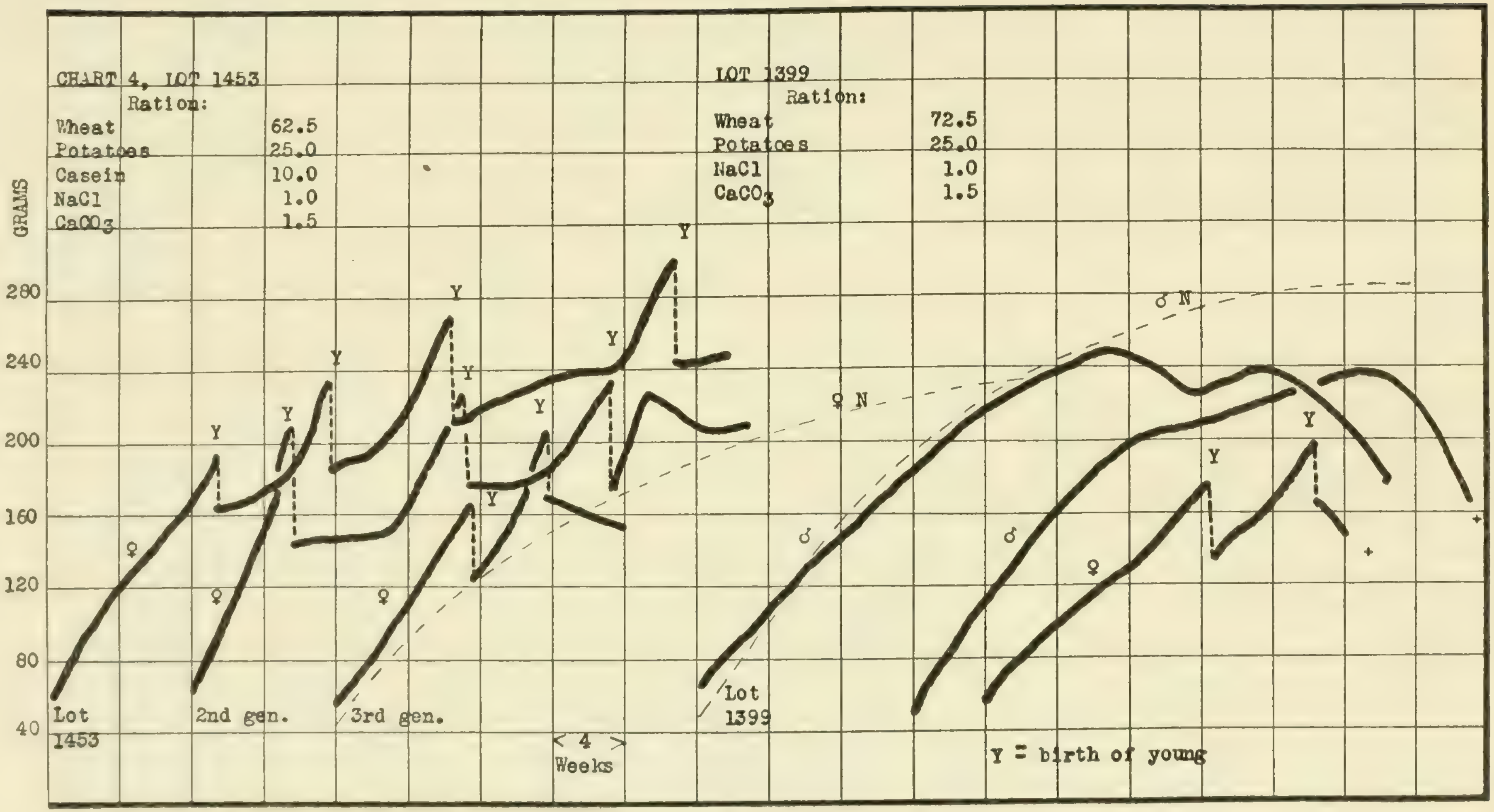
CHART 4, LOT 1453
Ration:
Wheat 62.5
Potatoes 25.0
Casein 10.0
NaCl 1.0
CaCO3 1.5
LOT 1399
Ration:
Wheat 72.5
Potatoes 25.0
NaCl 1.0
CaCO3 1.5
GRAMS
280
240
200
160
120
80
40
Lot 1453
2nd gen.
3rd gen.
Lot 1399
< 4 > Weeks
Y = birth of young
♀ N
♂ N
♀
♂
Y
+

sources is capable of inducing good growth and moderately high fertility is shown by a comparison with Lot 1408, Chart 5. The limiting factor in Lot 1399 is fat-soluble A. There were two females in this group. One had two litters (eleven young) but destroyed them soon after birth. The other remained sterile.

Lot 1453 had a diet similar to that of Lot 1399 except that it contained 10 per cent of casein to supplement its protein content. This exerted marked improvement in their growth, fertility, and longevity. It is of special importance to compare these curves with those of Lots 1399 and 1408. The diet of Lot 1399 is very much improved either by the addition of protein or fat-soluble A. This illustrates a principle which many investigators have failed to grasp; *viz.*, that all the other factors in an experimental diet must accurately be evaluated before judgment can safely be formed concerning the quality of a food or preparation in respect to a factor under investigation. For example, the diet of Lot 1453 appears to contain sufficient fat-soluble A. Actually it is distinctly below the optimum in this substance. It contains the same amount of fat-soluble A as does the diet of Lot 1399, but this deficiency is not apparent in Lot 1453 for some months because of the excellent quality in all other factors. Deficiency in fat-soluble A, however, became manifest in the lowered nutritive value of the milk and caused injury to the young, especially in the third generation.

Two females in this group had collectively fifty-two young (seven litters) and weaned forty-two of them. One second generation female had twenty young (three litters) and weaned eleven. One third generation female had eleven young (two litters) and weaned nine of them. The young were strong and vigorous in most cases. A few of the second litter obtained from a third generation female were, however, quite undersized and puny.

Chart 5.—The curve of Lot 1462, Period 1, illustrates the behavior of young rats fed a diet in which all the inorganic salts were furnished by a mixture of wheat, casein, and potatoes. No growth could take place on this diet even though all factors other than the inorganic elements were properly constituted. In Period 2 when calcium carbonate and sodium chloride were added growth took place at once and at the maximum rate possible.

There were three females in this group. They had together forty-five young (six litters) and successfully weaned them all.

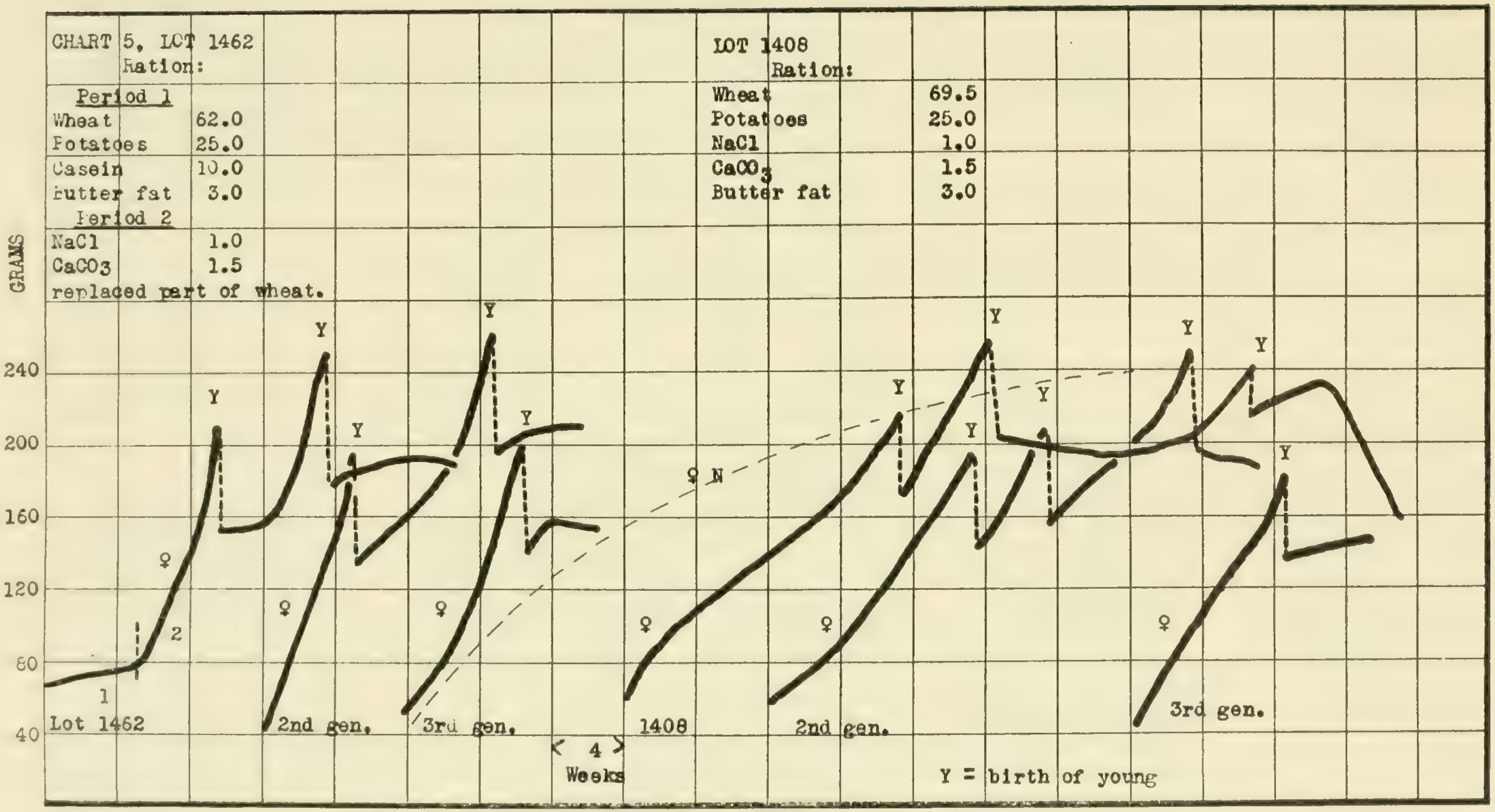
CHART 5, LOT 1462
Ration:
Period 1
Wheat 62.0
Potatoes 25.0
Casein 10.0
Butter fat 3.0
Period 2
NaCl 1.0
CaCO3 1.5
replaced part of wheat.
LOT 1408
Ration:
Wheat 69.5
Potatoes 25.0
NaCl 1.0
CaCO3 1.5
Butter fat 3.0
GRAMS
240
200
160
120
80
40
Lot 1462
1
2
2nd gen.
3rd gen.
1408
2nd gen.
3rd gen.
♀
♀ N
Y
< 4 >
Weeks
Y = birth of young

One second generation female was restricted to the family diet. She had two litters (sixteen young) and weaned them all. One third generation female had a litter of nine and reared them all. The young were always in excellent condition. This is an example of the success which we regularly see in successful growth and rearing of young when the mother's diet is satisfactory.

Lot 1408 derived all its protein from wheat and potato. The protein content of the diet was about 9 per cent, two-thirds of which was derived from wheat and one-third from potato. This amount of protein from these sources did not support growth at the maximum rate possible but the results indicate that there is some improvement in quality by combining proteins from these sources.

Two females in this group had twenty-one young collectively (four litters) and weaned eleven. One female in the second generation had eighteen young (three litters) and weaned eight of them. Two third generation females had fifteen young (two litters) of which twelve were weaned. The young in all these litters were small for their ages, and the nursing period was considerably prolonged in all cases in order to bring the young to a state of independence. Since these litters were not large in the first or second generation, it would seem probable that the failure of the young to thrive in a normal manner was due to faults in the quality rather than to lack of sufficient milk.

A comparison of the reproduction records of these groups (Lots 1462 and 1408) shows clearly that the infant mortality in the latter was the result of an inadequacy of the protein of the diet. On low protein diets, or diets containing protein of poor quality, we have regularly observed the necessity of a prolonged nursing period in order to fit the young to subsist on rations suitable for the adult.

Chart 6.—Lot 2150 illustrates a typical record of a group of rats fed from weaning time on a diet in which the protein was derived from peas and potatoes. Two-thirds of the protein was supplied by peas and the remainder by potato. The diet contained 9 per cent of protein (N × 6.25). Growth on this diet was approximately normal, showing that the mixed proteins of peas and potato at this plane of intake are far superior to pea proteins alone fed at this level (compare Lot 2376, Chart 1). Since dried potato is so

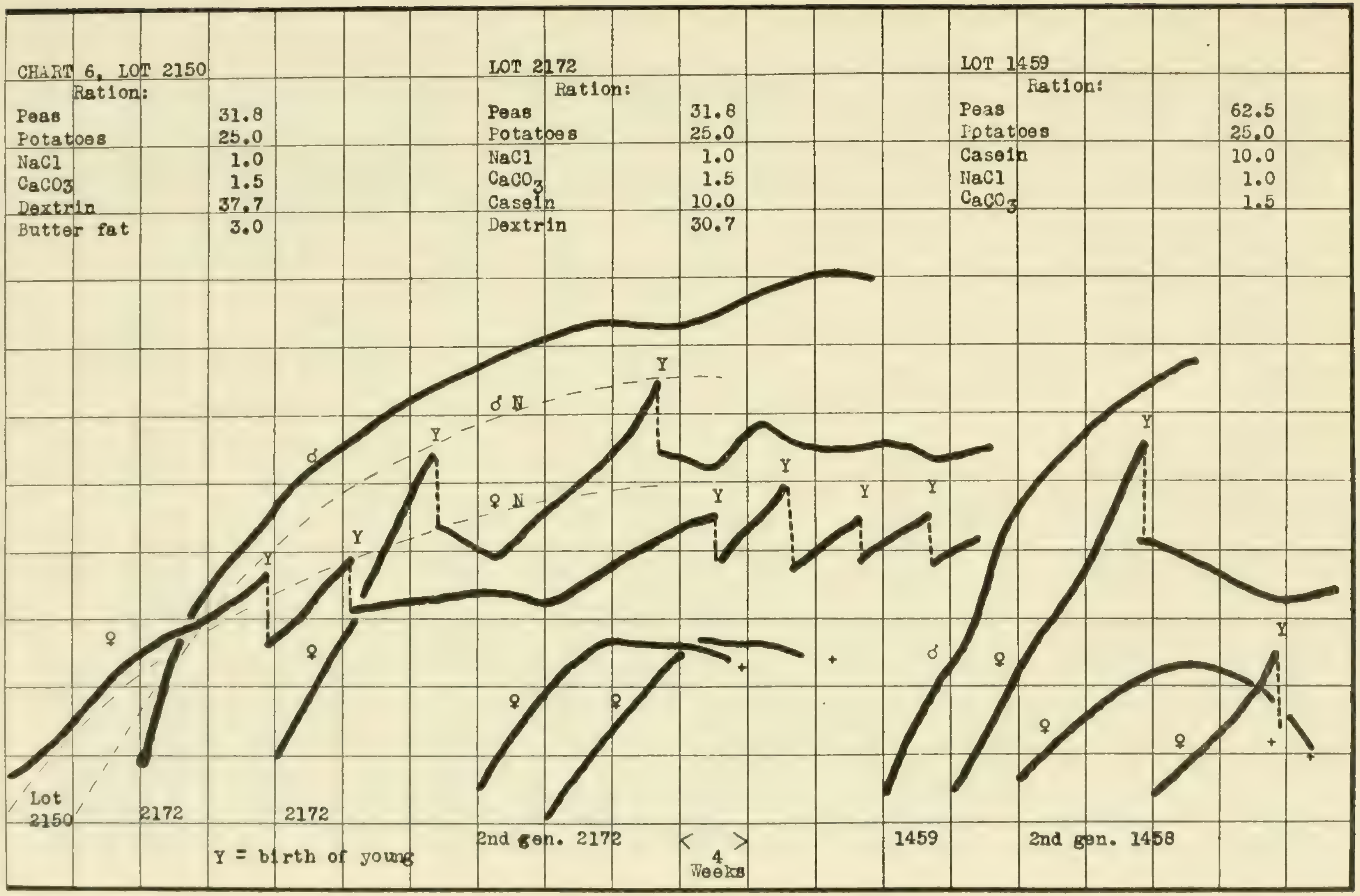
CHART 6, LOT 2150
Ration:
Peas 31.8
Potatoes 25.0
NaCl 1.0
CaCO3 1.5
Dextrin 37.7
Butter fat 3.0
LOT 2172
Ration:
Peas 31.8
Potatoes 25.0
NaCl 1.0
CaCO3 1.5
Casein 10.0
Dextrin 30.7
LOT 1459
Ration:
Peas 62.5
Potatoes 25.0
Casein 10.0
NaCl 1.0
CaCO3 1.5
GRAMS
360
320
280
240
200
160
120
80
40
♂
♀
♂ N
♀ N
Y
+
Lot 2150
2172
2172
2nd gen. 2172
1459
2nd gen. 1458
Y = birth of young
< 4 >
Weeks

low in protein it is not possible to make a direct comparison of these mixed proteins with potato protein alone. That this content of protein from these sources is below the optimum is shown by a comparison of Lots 2150 and 2172.

There were three females in this group. They had collectively six litters (eighteen young) but none was weaned. Two other litters were born but were destroyed by the mother before the number was ascertained. The rats in this group looked very old at about the age of 1 year.

Lot 2172 had a diet similar to that of Lot 2150 except that it contained casein but no butter fat. The growth records were remarkably good, notwithstanding the fact that all the fat-soluble A was derived from peas (31.8 per cent) and potato (25 per cent). One male reached a weight of 360 gm., and a female, a non-pregnant weight of 245 gm.

There were two females in the group. They had together twenty-three young (four litters) and weaned thirteen of them. The young were inferior in appearance, but did not develop xerophthalmia. One mother, however, developed inflamed eyes. Four second generation females were restricted to the food mixture but all died after being subjected to this diet for 4 to 5 months without having any young.

Lot 1459 was fed a diet similar to that of Lot 2172, except that it contained about double the amount of peas. The first generation fed this diet grew well, but the fertility was low and they aged very early. They were ready to die at about a year.

There were two females in this group. They had collectively nineteen young (three litters) and weaned nine of them. Two females in the second generation were restricted to the diet. One became pregnant, but died while giving birth to young. The other died at the age of 6 months. This ration contained a high protein content (about 27.5 per cent), more than half of which was from peas. Pea protein when fed at high levels apparently causes damage to animals.[3] This diet (Lot 1459) contained considerably more fat-soluble A and protein in its content of peas than did that of Lot 2172. All other factors were equally satisfactory or to the advantage (water-soluble B) in the case of Lot 1459, yet the nutrition of the latter group was distinctly inferior to Lot 2172. These results harmonize with the observation of Osborne and Mendel[4] that young rats fed purified pea protein as the sole source of this factor steadily declined.

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[3] McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1919, xxxvii, 287.

The records of Lot 1459 should be compared with those of Lot 1468 (Chart 7). The diets of these groups were similar but the latter contained more fat-soluble A, and this resulted in marked improvement in their condition.

Chart 7.—The rats of Lots 1414 and 1405 derived their protein entirely from peas and potatoes. The pea protein constituted about 88 per cent of the total. The protein content of these food mixtures was 17 to 18 per cent. Even at this high level of intake these proteins are inferior to a mixture derived from wheat and potato, as is shown by a comparison of these groups with Lot 1408 (Chart 5).

Lot 1414 contained two females. They had collectively three litters (nineteen young) and weaned all of them. One female of the second generation had one litter of three and weaned them. Some of the original group were kept on the diet more than 10 months but by 8 months they appeared old looking.

Lot 1405 was fed a diet closely similar to that of Lot 1414 except that it contained no butter fat. The inferiority of these animals as compared with Lot 1414 was due entirely to the lower intake of fat-soluble A.

A striking illustration of the danger of misinterpreting data obtained in nutrition experiments of this character is afforded by a comparison of four sets of records in these charts. Lot 2172 (Chart 6) derived all its fat soluble-A from 31.8 per cent of peas and 25 per cent of potato. The protein was enhanced by the addition of casein and the necessary inorganic salts were added to complete the mineral content. During the first year of life these animals appeared normal except for the high infant mortality. Lot 1459 (Chart 6) had more peas, the same amount of potatoes and casein, and the same salts. It, therefore, secured more protein and more fat-soluble A than Lot 2172, yet the development of Lot 2172 was much more satisfactory. If we did not have the records of Lot 2172 we should have made the statement from the results of Lot 1405 that peas are very low in fat-soluble A. How-

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[4] Osborne, T. B., and Mendel, L. B., *Z. physiol. Chem.*, 1912, lxxx, 307.

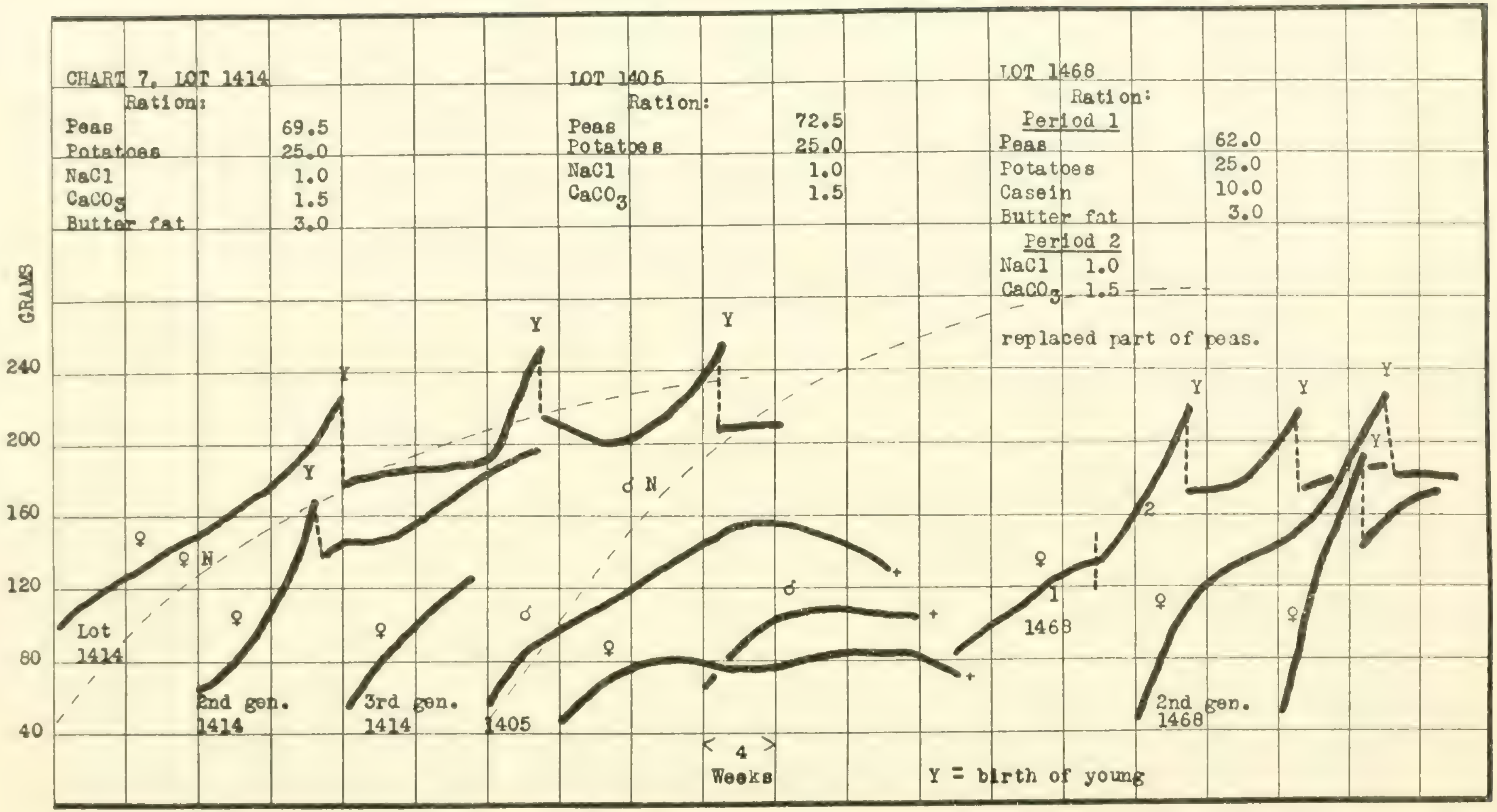
CHART 7. LOT 1414
Ration:
Peas 69.5
Potatoes 25.0
NaCl 1.0
CaCO3 1.5
Butter fat 3.0
LOT 1405
Ration:
Peas 72.5
Potatoes 25.0
NaCl 1.0
CaCO3 1.5
LOT 1468
Ration:
Period 1
Peas 62.0
Potatoes 25.0
Casein 10.0
Butter fat 3.0
Period 2
NaCl 1.0
CaCO3 1.5
replaced part of peas.
GRAMS
240
200
160
120
80
40
Y
♀
♀ N
♀
Lot 1414
2nd gen. 1414
♀
3rd gen. 1414
♂
1405
♂ N
♀
♂
+
+
+
< 4 >
Weeks
Y = birth of young
♀
1
1468
2
♀
2nd gen. 1468
♀

ever, when the protein content was improved with casein, as in Lot 2172, the fat-soluble A derived from 31.8 per cent of peas and 25 per cent of potato had more beneficial effect than more than twice this amount taken with the same food mixture containing protein of lower biological value. The addition of butter fat (fat-soluble A) to Lot 1405, however, also made them develop distinctly better, as is shown in Lot 1414.

Lot 1405 (Chart 7) was fed 72.5 per cent of peas and therefore more fat-soluble A than either Lot 2172 or Lot 1459 (Chart 6), yet because the casein was omitted in Lot 1405 the animals were very inferior. The protein and phosphorus content of Lot 2172 is lower than that of Lot 1405, yet the growth was much better in the former than in the latter. This was due to superiority in the quality of the protein in the diet of Lot 2172, and this factor was of sufficient biological significance to make a decided difference in the well being of the two groups. These illustrate border-line cases of malnutrition. They represent a type of diet in which a small variation of quality upward in one or another factor, as protein or salts, may so modify the early history of the experimental animals as to make a particular factor, *e.g.* fat-soluble A content, appear in one set of results to be adequate for the physiological needs of the animals, while a variation in the quality downward either in protein or salts, such variation remaining at the same time well within physiological limits, may entirely change the deductions with respect to the value of the constant factor fat-soluble A.

This same idea is also brought out in Chart 8, Lot 1454, and also in Chart 11, Lot 1451 (see discussion under these charts).

Lot 1468 in Period 1 was fed a diet of peas, potatoes, casein, and butter fat. The growth of the animals on this food was in no case more than half normal (Period 1). In Period 2 sodium chloride and calcium carbonate were added. This led to a prompt response with growth and reproduction. It appears probable from these results that there is less injurious effect of high pea protein ingestion when its quality is enhanced by a casein addition than when the same pea protein content is taken without such improvement.

This group contained two females. They had collectively fourteen young (two litters) and weaned them all. Two second generation females had collectively twelve young (a litter each) and weaned eleven. These mothers were discarded after weaning their young. These animals presented a dirty appearance and were badly stained with their own urine.

Chart 8.—Lot 2149 was fed a diet, the protein of which was derived entirely from rolled oats and potato. It contained 9 per cent of total protein (N × 6.25), two-thirds of which was furnished by oats and one-third by potato. Other factors than protein were satisfactorily supplemented. Growth on this diet was somewhat below the optimum, but the protein derived from this combination is of better quality than a similar amount of oat protein alone. This combination does not possess so high a biological value as does a protein mixture from wheat and potato in the same proportions. This is illustrated by Lot 1408 (Chart 5).

Three females grew up on this diet. They had collectively thirty-one young (four litters) but none was weaned. The males were apparently in good condition at 7 months of age, but the females presented a poorer appearance. Those young which were not killed by the mother grew so little while nursing that it is evident that the nutritive value of the milk was low.

Lot 2173 derived all its fat-soluble A from rolled oats and potato. In other respects this diet was comparable with Lot 2149. The protein in the diet of Lot 2173 was enhanced by the addition of casein.

Three females grew up on this diet. One female had two litters, another, one litter, collectively eighteen young, and weaned but four of them. A third female became pregnant but died while giving birth to her young The young were in a very poor condition. Two daughters of these mothers were restricted to the diet but never had any young. All these animals aged early. Their span of life was only 5 to 7 months.

Lot 1454 presents remarkable growth curves on a diet which all previous experience indicated to be very poor in fat-soluble A. All of this factor was derived from 62.5 per cent of rolled oats and 25 per cent of cooked dried potato. One male reached a weight of over 400 gm. Two females each had three litters, collectively forty-seven young, and weaned twenty-six. One daughter grew up on the diet and had two litters but lost them all during the first few days. These young were in poor condition and developed xerophthalmia in some cases. At the age of 11 months the animals appeared very old, but autopsy revealed nothing pathological.

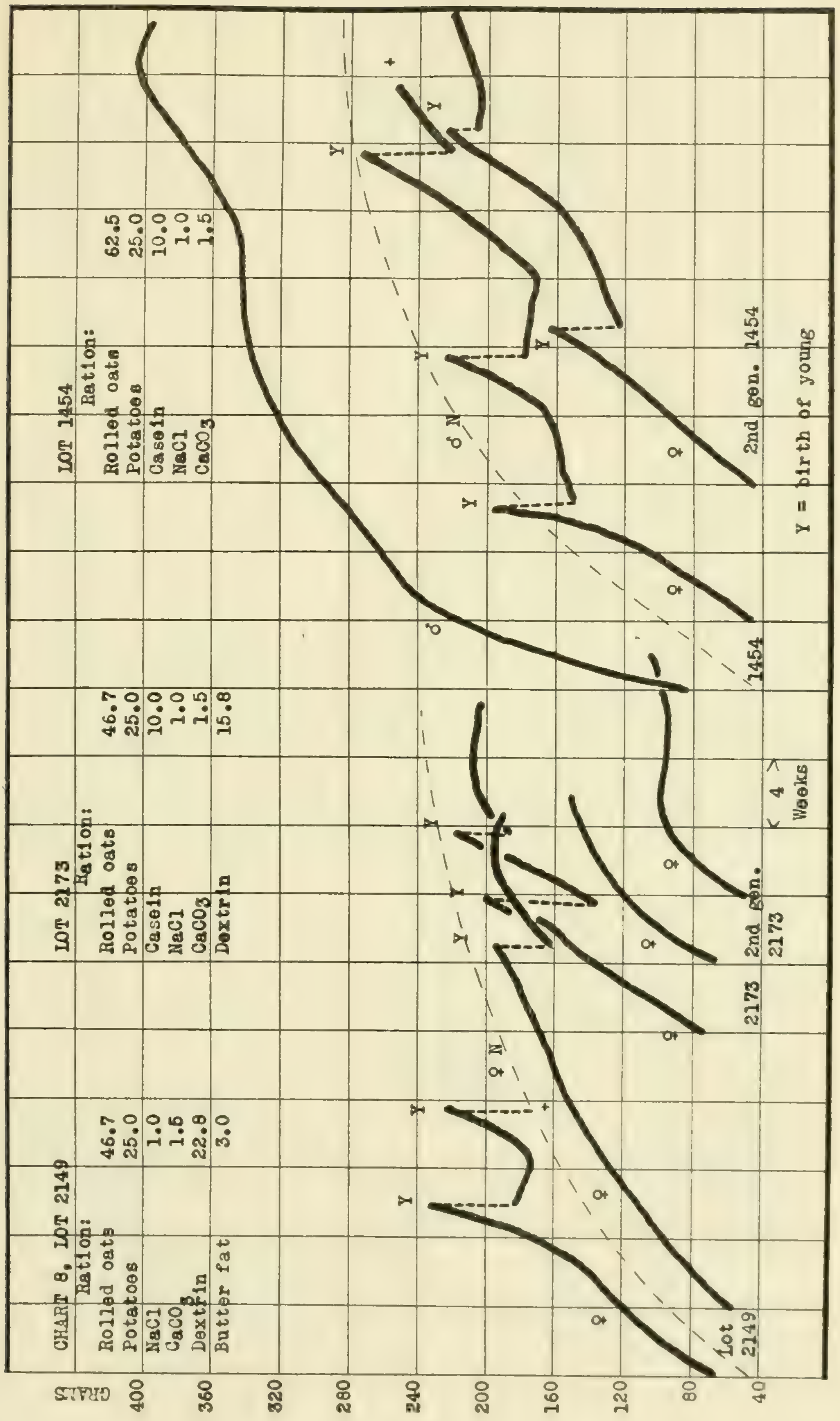
CHART 8, LOT 2149
Ration:
Rolled oats 46.7
Potatoes 25.0
NaCl 1.0
CaCO3 1.5
Dextrin 22.8
Butter fat 3.0
LOT 2173
Ration:
Rolled oats 46.7
Potatoes 25.0
Casein 10.0
NaCl 1.0
CaCO3 1.5
Dextrin 15.8
LOT 1454
Ration:
Rolled oats 62.5
Potatoes 25.0
Casein 10.0
NaCl 1.0
CaCO3 1.5
GRAMS
400
360
320
280
240
200
160
120
80
40
Lot 2149
2173
2nd gen. 2173
1454
2nd gen. 1454
♀
♂
♀ N
♂ N
Y
< 4 > Weeks
Y = birth of young

Our experience in studying the dietary properties of the oat kernel has demonstrated it to be the poorest of the cereal grains in fat-soluble A. Steenbock and Gross report the white potato to be an inadequate source of this factor.[5] In interpreting the value of this diet with respect to fat-soluble A, Lot 1454 (Chart 8) and Lots 1409 and 1400 (Chart 9) should be compared. On the diet of Lot 1400 but little growth could take place, and xerophthalmia developed. Lot 1409, whose diet differed from that of Lot 1400 only in containing butter fat, was able to grow and reproduce. This was due specifically to the increased content of fat-soluble A. Lot 1454, whose diet differed from that of Lot 1400 only in that its protein content was improved by the addition of casein was also able to grow normally and reproduce and rear some young. The infant mortality was higher than that of Lot 1409. The content of fat-soluble A was the same in the diets of Lots 1400 and 1454. Such experimental results as these show the fallacy of deductions such as have been made by Hess and Unger[6] who fed infants during several months on a diet somewhat low in fat-soluble A but of excellent quality in other respects, and concluded that the factor fat-soluble A is not of great importance in practical human nutrition. Judgment on this matter must be based on a full appreciation of the difference between border-line malnutrition with its attendant grave dangers from infections or unfavorable reaction to any chance modification of the diet so as to reduce its quality in any way.

Chart 9.—Lot 1409 was fed a diet in which the protein was all derived from rolled oats and potato. The content of protein in the food mixture was about 12.5 per cent, about 16 per cent of this being derived from potato, and 84 per cent from oats. All other factors were adequately supplemented.

The rats which were confined to this diet never grew to the normal adult size. There were two females in this group. They had collectively eighteen young (four litters) but only two were weaned. The mothers destroyed the young. One of these rats died during the birth of her third litter. One second generation female grew up on the family diet and had fifteen young (two litters) and

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[5] Steenbock, H., and Gross, E. G., *J. Biol. Chem.*, 1919, xl, 501.

[6] Hess, A. F., and Unger, L. J., *J. Am. Med. Assn.*, 1920, lxxiv, 217.

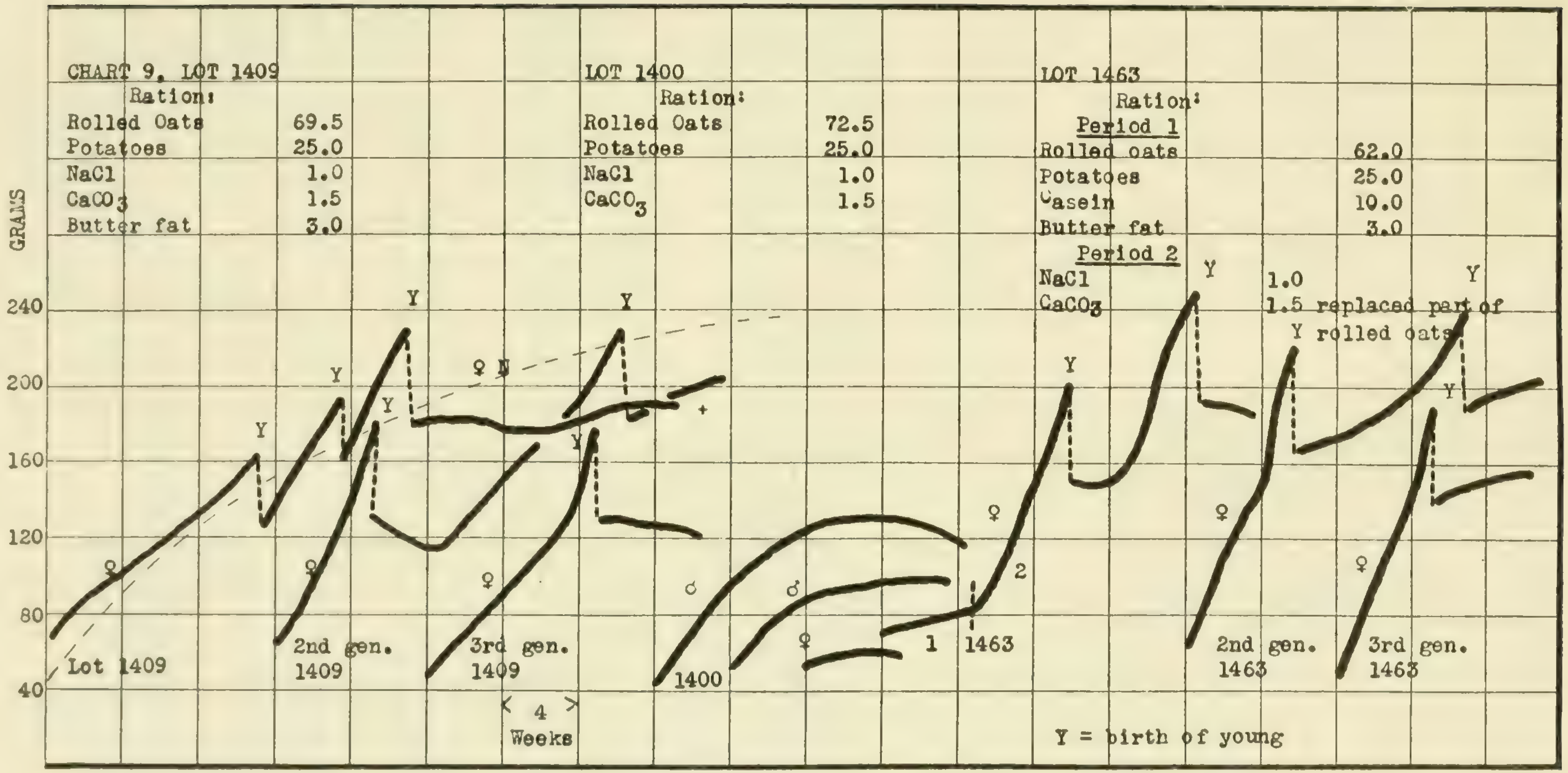
CHART 9. LOT 1409
Ration:
Rolled Oats 69.5
Potatoes 25.0
NaCl 1.0
$CaCO_3$ 1.5
Butter fat 3.0
LOT 1400
Ration:
Rolled Oats 72.5
Potatoes 25.0
NaCl 1.0
$CaCO_3$ 1.5
LOT 1463
Ration:
Period 1
Rolled oats 62.0
Potatoes 25.0
Casein 10.0
Butter fat 3.0
Period 2
NaCl 1.0
$CaCO_3$ 1.5 replaced part of rolled oats
GRAMS
240
200
160
120
80
40
Y
♀
♂
♀ N
+
1
2
Lot 1409
2nd gen. 1409
3rd gen. 1409
1400
1463
2nd gen. 1463
3rd gen. 1463
< 4 > Weeks
Y = birth of young

weaned thirteen of them. One third generation female had one litter of six and successfully weaned them.

Lot 1400 was fed a diet like that of Lot 1409 except that it did not have a supplemental addition of fat-soluble A. Growth on this diet was slow and incomplete. The rats all died in 4 to 5 months after being placed upon the ration. Xerophthalmia developed in three of the four rats confined to this food mixture.

This diet with added fat-soluble A is capable of inducing nearly normal growth and supports reproduction and rearing of some young (Lot 1409). This ration with added protein has induced the maximum amount of growth in rats and supported reproduction and rearing of some young, although the infant mortality was high (Lot 1454, Chart 8). This illustrates the fact that one is not justified in drawing conclusions concerning the content of fat-soluble A in a foodstuff without furnishing evidence that all factors in the diet are accurately evaluated.

Lot 1463 was fed in Period 1 a diet consisting of rolled oats, potatoes, casein, and butter fat. The inorganic content of the diet was entirely derived from these sources. Practically no growth could take place on this food. In Period 2 sodium chloride and calcium carbonate were added. This led to marked response with growth to the full adult size.

Three females in this group had collectively thirty-eight young (six litters) and weaned thirty-six. One second generation female was restricted to the diet. She had fifteen young (two litters) of which fourteen were weaned. One third generation female had a litter of nine and weaned them all. These young were in all cases in excellent nutritive condition.

A comparison of the reproduction records and infant mortality of Lot 1463 with those of Lot 1454 (Chart 8) shows clearly the beneficial effects of a liberal supply of fat-soluble A. This is shown by a mortality among the young of 45 per cent in Lot 1454 in the first generation and of 100 per cent in the second generation.

In Lot 1463, 95 per cent of the young were weaned. The appearance, size, and condition of the young of these two groups presented as marked a contrast as did the infant mortality.

Chart 10.—Lot 1406 derived all its protein from 69.5 per cent of maize and 25.0 per cent of potatoes. All other factors were

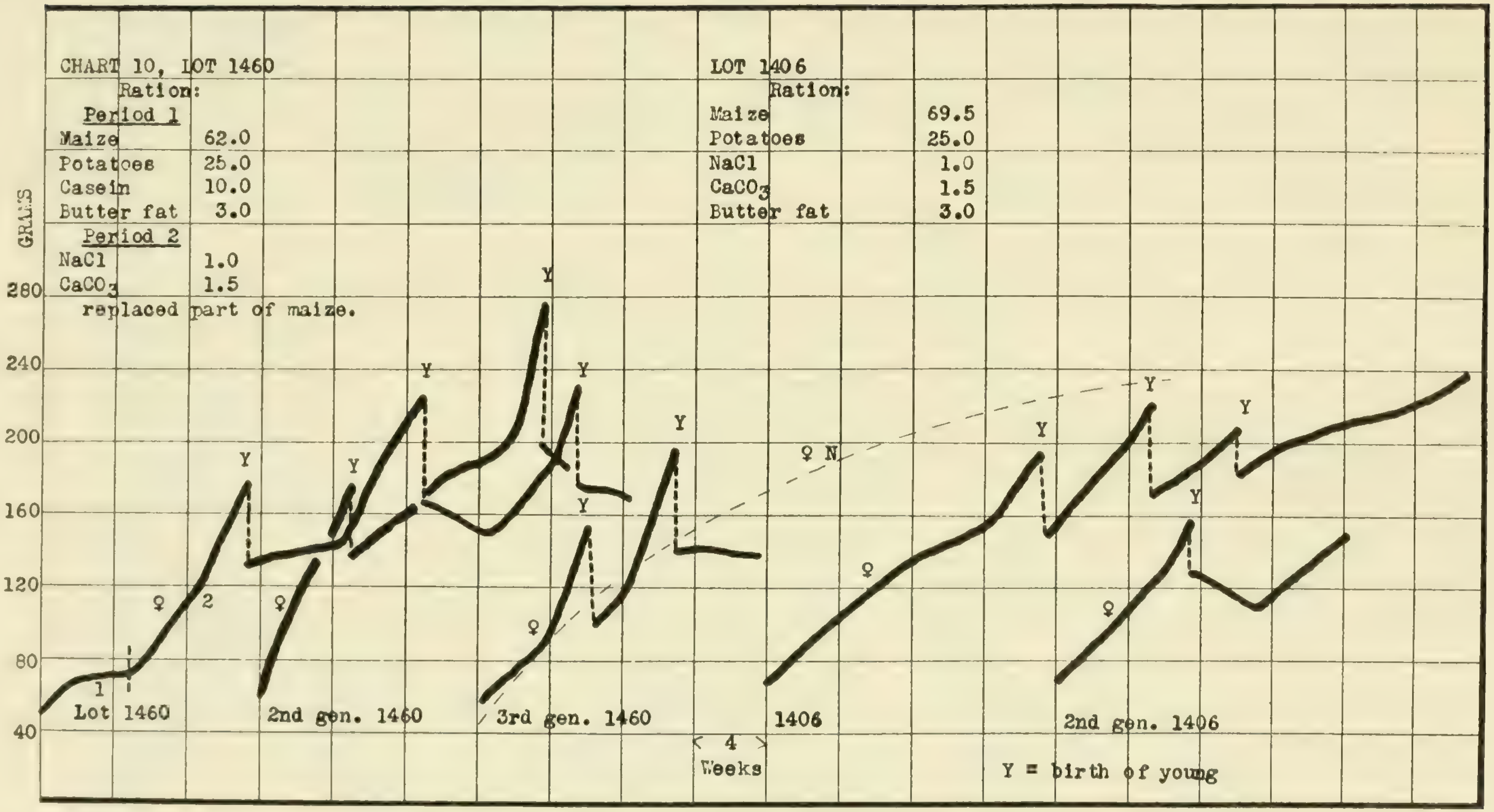
CHART 10, LOT 1460
Ration:
Period 1
Maize 62.0
Potatoes 25.0
Casein 10.0
Butter fat 3.0
Period 2
NaCl 1.0
CaCO3 1.5
replaced part of maize.
LOT 1406
Ration:
Maize 69.5
Potatoes 25.0
NaCl 1.0
CaCO3 1.5
Butter fat 3.0
GRAMS
280
240
200
160
120
80
40
Y
Y
Y
Y
Y
Y
Y
Y
Y
Y
Y
Y
♀
♀
♀
♀
♀
♀
♀ N
1
2
Lot 1460
2nd gen. 1460
3rd gen. 1460
1406
2nd gen. 1406
< 4 >
Weeks
Y = birth of young

satisfactorily adjusted by suitable additions. Growth was somewhat retarded because of the quality in the protein factor.

Three females grew on this diet. They had collectively thirty-five young (six litters) and weaned eleven of them. Two second generation females were restricted to this diet. One had a litter of two young and after a long nursing period weaned them in poor condition. The other remained sterile. The animals in this group were always somewhat undersized and of poor appearance.

Lot 1460 in Period 2 had a diet like that of Lot 1406 except that it contained 10 per cent of casein. In Period 1 no inorganic salts were added. Without these growth was suspended, but with them the animals grew rapidly. This shows that in Lot 1406 the quality and amount of protein was the limiting factor.

Three females grew up on this diet. They had collectively fifty young (seven litters) and weaned forty-eight of them in good condition. One second generation female had seventeen young (two litters) and weaned fifteen of them. One third generation female had fifteen young (two litters) and weaned seven. The young were vigorous and active but their coats were not as glossy and well kept as those of other rats which we have observed. The contrast between these young and those of Lot 1406, on a lower protein plane, was very marked.

Chart 11.—Lot 1397 derived both protein and fat-soluble A entirely from maize and potato. The maize used in these experiments was of the yellow variety. A comparison of these growth curves with those of Lot 1451 (Chart 11) shows that notwithstanding the high content of yellow maize in this food mixture, protein was the first limiting factor in preventing normal development. Lack of sufficient fat-soluble A was, however, a very important deficiency in this diet, as shown by comparing the curves of Lot 1397 with those of Lot 1406 (Chart 10).

There was more fat-soluble A in the diet of Lot 1397 than in that of Lot 1451, yet the satisfactory content of protein in the latter diet made it appear, in the absence of a complete series of biological tests, to contain a satisfactory amount of this factor. Although certain yellow maizes doubtless contain more fat-soluble A than certain white varieties it seems to us that there has been some exaggeration of the value of yellow maize as a source of this

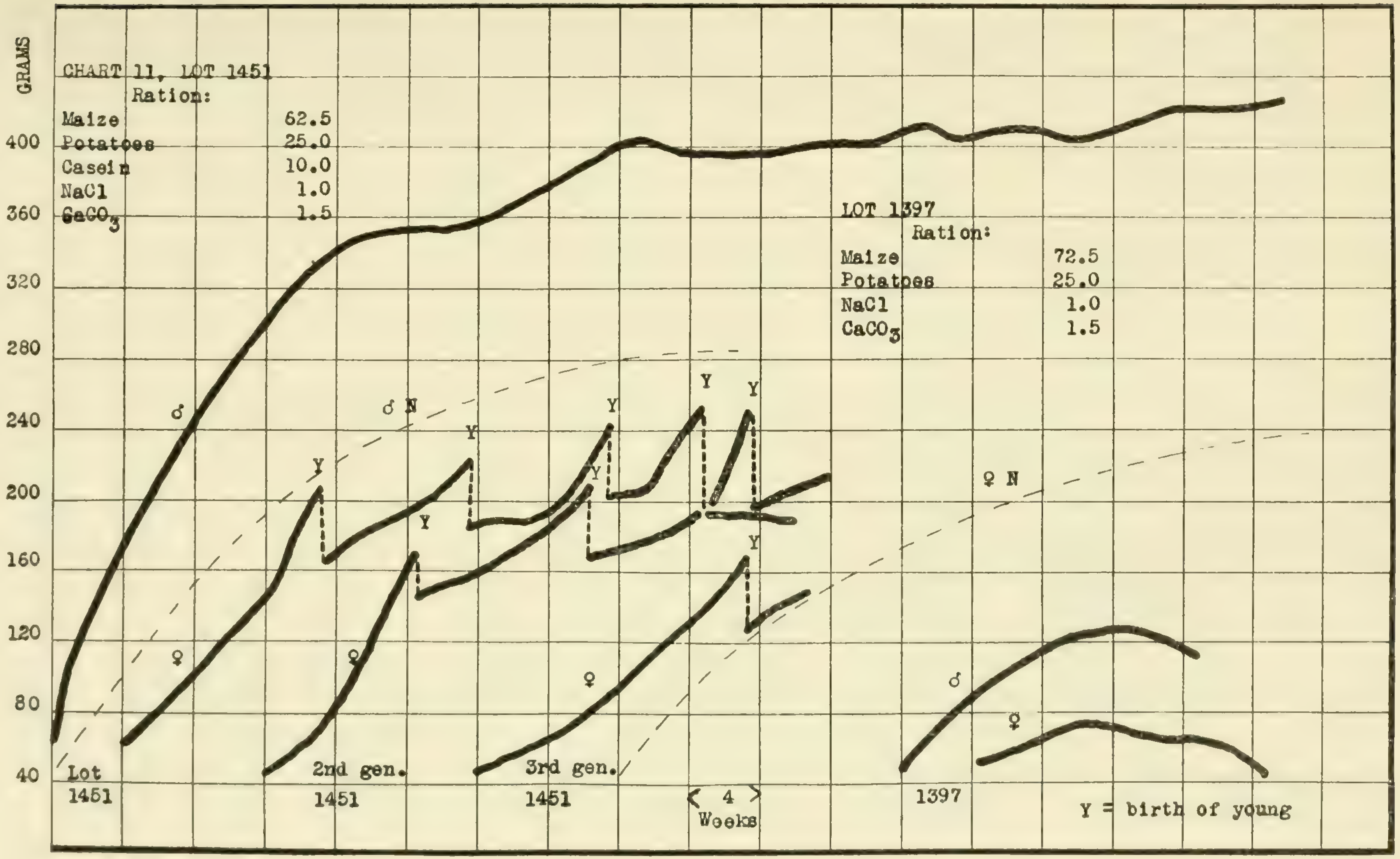
CHART 11, LOT 1451
Ration:
Maize 62.5
Potatoes 25.0
Casein 10.0
NaCl 1.0
$CaCO_3$ 1.5
LOT 1397
Ration:
Maize 72.5
Potatoes 25.0
NaCl 1.0
$CaCO_3$ 1.5
GRAMS
400
360
320
280
240
200
160
120
80
40
♂
♀
♂ N
♀ N
Y
Lot 1451
2nd gen. 1451
3rd gen. 1451
1397
< 4 > Weeks
Y = birth of young

factor.[7] If we were limited to experiments with diets of the type fed Lots 1451 (Chart 11) and 1454 (Chart 8) we could easily have fallen into the error of interpreting the results as indicating that diets containing 60 to 70 per cent of yellow maize or even rolled oats supplemented with potato to the extent of 25 per cent of the food mixture would prove satisfactory in their content of fat-soluble A. More carefully planned experiments eliminate the danger of error in studies of this nature.

Lot 1451 contained two females. They had collectively sixty-four young (eight litters) and weaned fifty-nine. Two second generation females had five litters (twenty-seven young) and weaned twenty-two. Two third generation females had collectively eleven young (two litters) and weaned ten. All the young were in good condition, but in a few cases the hair was short and silky in appearance.

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[7] Steenbock, H., and Boutwell, P. W., *J. Biol. Chem.*, 1920, xli, 81.

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SUPPLEMENTARY PROTEIN VALUES IN FOODS.

IV. THE SUPPLEMENTARY RELATIONS OF CEREAL GRAIN WITH CEREAL GRAIN; LEGUME SEED WITH LEGUME SEED; AND CEREAL GRAIN WITH LEGUME SEED; WITH RESPECT TO IMPROVEMENT IN THE QUALITY OF THEIR PROTEINS.

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Those who have discussed the world's food supply during the past few years have talked in great measure about the cereal grains as the most important and economical source of human food. Indeed, for many years the cereals and legume seeds have been increasingly cultivated both in Europe and America to meet the needs of their rapidly increasing populations. Bread grains have actually become the staff of life of a large part of the human family. It is now recognized by students of nutrition that the cereal grains individually and collectively are incomplete foods.[1] The war has taught through tragic experience that children cannot be kept alive for a very long period on such a food supply, although the requisite calories and energy may be supplied. The history of scurvy furnishes numerous examples of the dangers attending the restriction of the diet of adults to cereal products, legume seeds, and meats. Nevertheless, statisticians and persons in charge of feeding large groups of people in armies, prisons, asylums, hospitals, and labor camps, still in many instances show lack of appreciation of the dangers of limiting people to certain types of restricted diets.

It was natural that in the early efforts to discover the fundamental truths regarding the nutritive requirements of mammals, investigators should have attached much importance to the rate of growth during limited periods by young animals fed diets of different types. It was spectacular to observe complete failure in one case on a diet with which a chemist could find nothing amiss, and successful growth for weeks or months on another diet which according to older views should have been no more satisfactory than the first. With advancing knowledge, however, it became possible to interpret the cause of failure or success with any given diet, and new goals were set up by those who were able to make progress in nutrition studies. In our laboratory we have for several years sought to approach the solution of the problem of what constitutes the optimum diet for the purpose of promoting growth, of supporting highest fertility, greatest success in rearing young, and of preserving for as long a period as possible the characteristics of youth, and of extending to the extreme limit the span of life. With this objective we have come, as the result of much experience, to question whether the extension of the use of cereal grains in the diet of man has not already passed the limit of safety. At least it is more necessary now than formerly that the remaining components of the diet should be chosen with knowledge and care in order to correct the deficiencies of the cereal, tuber, and muscle meat mixture, or the bread, potato, and meat type of diet which has become so prominent a feature of the nutrition of many American and European families at the present time. It has been already pointed out that the cereal grains, legume seeds, and muscle tissue meats are too poor in calcium, sodium, chlorine, and fat-soluble A to meet the physiological requirements of a mammal. Mixtures of these in any variety are little, if any, better sources of the essential mineral elements or of fat-soluble A than are the individual foods themselves.[2]

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[1] McCollum, E. V., The newer knowledge of nutrition, New York, 1918. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1920, xli, 275.

Primitive man ate everything he could secure which was edible. His animal food included the flesh of such game as he could catch, and also fish, eggs, birds, shell-fish, insects, etc. Among the vegetable products which he doubtless ate were fruits, berries, fleshy roots, nuts, and a few other seeds of plants, among which were the seeds of those grasses which have since been developed into our cereal crops. There are relatively few regions where nuts are sufficiently abundant to furnish a regular article of diet for a sparse population during even a few months in the year, and the supply of cereal grains was even more inadequate. The cereal grains are the seeds of several grasses. In a country where no agriculture was practised, grasses would be cropped by grazing animals and the development of seed greatly interfered with. As Huntington points out, extensive agriculture was impossible until after animals were domesticated.[3] Such seed as was produced was born on isolated and scattered stems, and would be difficult to harvest in appreciable quantities. One exception was the wild rice plant which grew in the water and was therefore protected to some extent from grazing animals. It was more abundant in certain places than the seeds of any land grasses were likely to be in the unmolested fruiting condition. Rice was therefore harvested from very early times in Asia and in some of the northern states of America. Even in these favored regions of shallow lakes and rivers, however, rice never formed a principal constituent of the diet of the Indians, but only an adjuvant in the fall and early winter. Grass seeds of the type of the cereal grains are always eagerly sought for at ripening by birds, and the harvest time would naturally be short. Maize was never a prominent article of diet among the Indians, but only served to vary their otherwise carnivorous food supply. All the higher apes eat more or less of tender leaves which have mild flavors. After man reached a stage of development where food was regularly cooked he was able to eat coarse vegetables of the leafy type in greater variety and in larger amounts than when he had to eat them raw, because of the difficulty of digesting some of them. Pot-herbs early became a regular part of the diet of man as he advanced toward civilization. They are today the outstanding feature of the diet of the Chinese and Japanese.

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[2] McCollum, E. V., Proceedings of the Institute of Medicine of Chicago, 1920, iii, 13.

The great increase in the consumption of cereal grains in various forms as flours, corn-meal, corn grits, rolled oats, and in the form of the many breakfast foods found on the market as human food is an incident in connection with the development of modern industry, and the change from a rural to an urban life. This has forced a great part of the population to depend upon the remainder for food while they operate the machinery of industry. The land yields calories and protein in greatest abundance when farmed to cereals and leguminous plants, rather than when used for the production of milk or meat. This type of farming has therefore been encouraged, and wheat and maize production has been stimulated year by year to higher and higher levels until the world's capacity has nearly been reached in this respect. This great consumption of grains has changed the character of the diet profoundly from what it has ever been before in human history, and in a manner which tends to undermine the vitality. The national dietetic sin of America and many parts of Europe has grown to be close adherence to a meat, bread, and potato diet, or other foods which have similar dietary properties. When it is remembered that the cereal grains are now all but universally decorticated and degerminated for the purpose of producing products which can be kept without commercial hazard, and that these are decidedly poorer in their dietary properties than are the seeds from which they were milled, the situation can easily be appreciated. The diet of cereals, muscle tissue meats, and tubers (the meat, bread, and potato type) is not satisfactory for the nutrition of man or animals, and is of distinctly poorer quality when highly milled products are used in abundance.[4]

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[3] Huntington, E., Civilization and climate, New Haven, 1916.

We wish to emphasize in connection with this study of the manner in which the cereal grains supplement, or rather fail to supplement, the proteins of other cereal grains and legume seeds, the *fact that the widespread use of this class of foods represents an innovation in man's diet, and one which is not for the best.* The cereals may well be used as articles of human food, but it is wiser to utilize more of the land for the extension of the dairy industry in order to increase the supply of milk and other dairy products, than to seek to extend as far as possible the production of crops which yield the greatest returns in such food units as the chemist has long recognized, but which fall short of the requirements of mammals in respects which we have but recently been able to appreciate.

When one studies the charts described in this paper it is difficult to avoid the conclusion that a diet composed too largely of cereal products, and not satisfactorily supplemented by foods which correct their deficiencies, will tend to lower the vitality and promote the early development of senile characters. We cannot agree with Daniels and Nichols that the consumption of legume seeds such as the soy bean should be increased.[5] It is much better to use these seeds for feeding dairy cows, for their deficiencies can be made good by the latter through the consumption of forage plants, and used as a source of milk formation. Milk forms the most satisfactory corrective food to make good the deficiencies of the cereal, muscle meat, and tuber diet now in such widespread use.

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[4] McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1919, xxxviii, 113.

SUMMARY.

The charts presented in this paper bring out the following facts: Earlier observations on the inferior character of the proteins of the legume seeds have been confirmed. It is further shown that protein mixtures derived from two such seeds, the selection being made from navy or soy beans or peas, are little, if any, better than the proteins of the individual seeds, which compose the mixture when fed as the sole source of nitrogen.

Two cereal grains when combined fail to form a protein mixture which is markedly superior to the same amount of protein from a single grain for the nutrition of the rat.

In certain instances the improvement in the quality of the proteins is decidedly great when a cereal grain is supplemented with a legume seed. Conspicuous examples of such enhancement of proteins are wheat and navy beans (Chart 12), and wheat and peas (Chart 13).

Table 1 presents the records of the weights of several litters of young rats, the mothers of which were confined to diets which were essentially comparable in quality in all factors other than protein (Lots 2369, 2365, 2370, and 1236). The rate of growth of the nursing young served as an index to the extent to which the protein moiety of the food mixture met the needs of the mother for her own maintenance and for milk production.

Lot 2369 should be contrasted with Lot 2069. Each litter contained four young. Lot 2369 derived its protein (9 per cent) from maize and peas. Growth was retarded, indicating that the quality of the milk was below the optimum. Lot 2069 derived its

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[5] Daniels, A. L., and Nichols, N. B., *J. Biol. Chem.*, 1917, xxxii, 91.

TABLE I.

This table shows typical records of nursing mothers on diets containing 9 per cent of protein from different sources, as contrasted with others on diets equally well constituted in respect to all other factors and containing higher protein contents of good quality.

Lot No.	Weight of ♀ when young were born.	Weight of ♀ 10 to 18 days later.		Weight of ♀ when through nursing.		No. of young.	Weight.						Source and content of protein.
	gm.	*days*	*gm.*	*days*	*gm.*		*days*	*gm.*	*days*	*gm.*	*days*	*gm.*	
2369	144	18	150	46	120	4	18	71	31	107	46	123	Maize 6 per cent protein, peas 3 per cent protein (see Chart 9).
2069	215	10	232	22	205	4	10	100	22	197			Degerminated cereals, peas beans, steak, and cabbage (18.2 per cent protein).
2365	173	18	157	45	146	5	18	70	30	102	45	210	Barley 6 per cent protein, navy beans 3 per cent protein (see Chart 8).
2370	192	12	170	37	170	5	12	74	33	160	37	175	Wheat 6 per cent protein, peas 3 per cent protein.
2153	175	15	182	28	192	5	15	137	28	277			Degerminated cereals, peas, beans, steak (19 per cent protein).
2370	177	20	145	44	175	7	12	81	33	167	44	250	Wheat 6 per cent protein, peas 3 per cent protein (see Chart 13).
1236	142			40	146	7	16	133	30	286	40	544	Steak 50 per cent, salts and butter fat, 35 per cent protein.

Lot 2069.	
Bolted flour	30.0
Corn-meal	15.5
Rice	8.0
Rolled oats	8.0
Peas	8.0
Navy beans	8.0
Round steak (cooked)	10.0
Cabbage (dry)	10.0
NaCl	1.0
$CaCO_3$	1.5

Lot 2153.	
Bolted flour	30.0
Corn-meal	19.5
Rice	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Round steak (cooked)	10.0
NaCl	1.0
$CaCO_3$	1.5

Lot 1236.	
Round steak (cooked)	50.0
NaCl	1.0
KCl	1.0
$CaCO_3$	1.5
Dextrin	43.5
Butter fat	3.0

protein from a variety of sources, and the amount was approximately double that in the diet of Lot 2369. These young were more than 25 per cent heavier at 10 days of age than those of Lot 2369 were at 18 days.

The nursing mother in Lot 2365 derived her protein (9 per cent) from barley and navy beans. The young nursing this mother fell far behind the growth of those which nursed a mother of Lot 2370, which derived its protein (9 per cent) from wheat and peas. The former litter of five young at 18 days weighed collectively 70 gm. The latter at 12 days weighed 74 gm. This difference in the nutrition of these nursing young was solely due to the difference in quality of protein in the two food mixtures. Other equally interesting comparisons can be seen in the table.

Lots 2069 and 2153 received 18 and 19 per cent of protein, respectively, and from a variety of sources. Their diets were, however, decidedly below the optimum in their content of fat-soluble A, and for this reason would not serve to maintain the vitality of a family through successive generations. A further discussion of this type of diet and its effect on quality of milk secreted and on the span of life will be given in a later paper.

Chart 1.—The curves presented in this chart are typical of groups of rats which were fed from an early age on diets which derived their protein contents from: (1) peas; (2) soy beans; (3) peas and navy beans; and (4) soy beans and navy beans. In each case the content of protein in the diets was 9 per cent. Lot 2367 derived two-thirds of its protein supply from peas and the remainder from navy beans. Lot 2366 derived two-thirds of its protein from soy beans and one-third from navy beans. Deficiencies in the

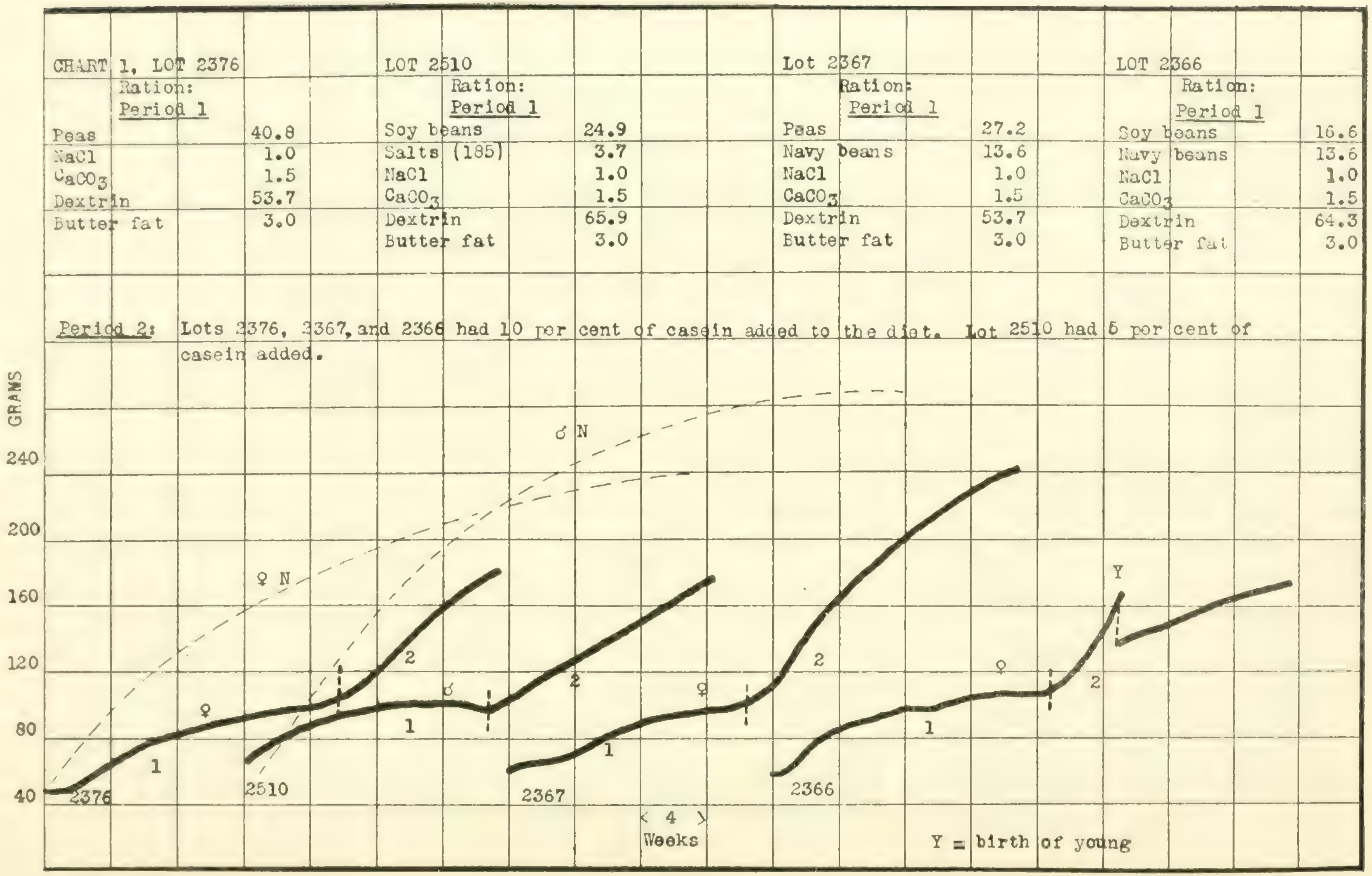

CHART 1, LOT 2376

Ration: Period 1	
Peas	40.8
NaCl	1.0
$CaCO_3$	1.5
Dextrin	53.7
Butter fat	3.0

LOT 2510

Ration: Period 1	
Soy beans	24.9
Salts (185)	3.7
NaCl	1.0
$CaCO_3$	1.5
Dextrin	65.9
Butter fat	3.0

Lot 2367

Ration: Period 1	
Peas	27.2
Navy beans	13.6
NaCl	1.0
$CaCO_3$	1.5
Dextrin	53.7
Butter fat	3.0

LOT 2366

Ration: Period 1	
Soy beans	16.6
Navy beans	13.6
NaCl	1.0
$CaCO_3$	1.5
Dextrin	64.3
Butter fat	3.0

Period 2: Lots 2376, 2367, and 2366 had 10 per cent of casein added to the diet. Lot 2510 had 5 per cent of casein added.

mineral and fat-soluble A content of the seeds were corrected by suitable additions, and accordingly the character of the growth curves depended on the quality of the protein.

These records show that combinations of proteins of peas and navy beans or combinations of proteins of soy beans and navy beans, are not superior in their biological values to the proteins of any one of these legume seeds when fed as the sole source of protein in diets of closely comparable composition in all respects other than the source of protein.

Of the females in Lot 2366 only one ever had any young. This rat had a litter of four which were destroyed soon after birth.

Chart 2.—The curves shown in this chart represent typical growth records of several groups of rats fed on diets deriving their proteins in each case from two legume seeds. The combinations include navy beans and peas; soy beans and peas; and navy beans and soy beans. Lot 2368 contained navy bean proteins as the sole source of nitrogen in a diet otherwise comparable in all respects to the diets of the animals whose curves are shown in the chart.

In Period 2, 10 per cent of casein was added to the diets. In every case this led to a marked response with growth, but doubtless at a slower rate than would have been the case if the animals had not suffered a long period of stunting.

It is a most remarkable fact that the legume seeds when combined with each other do not form protein mixtures which are superior to the proteins of the individual seeds themselves. This is, apparently, to be explained on the assumption that a certain amino-acid which is present in such small amount as to be the limiting factor in determining the biological value of the proteins of these seeds, is the same in each of the legume seeds used in these experiments. Otherwise, it seems that a supplementary effect should have been observed in some of these combinations. It is by no means demonstrated that all the indispensable amino-acids have been identified, but if one may judge from the recorded data relating to yields of various amino-acids it seems suggestive that the low content of cystine yielded by all legume proteins may be the explanation for their failure to enhance each others values when combined.[6] We shall later discuss experimental data shedding light on this point.

[6] Sure, B., *J. Biol. Chem.*, 1920, xliii, 443.

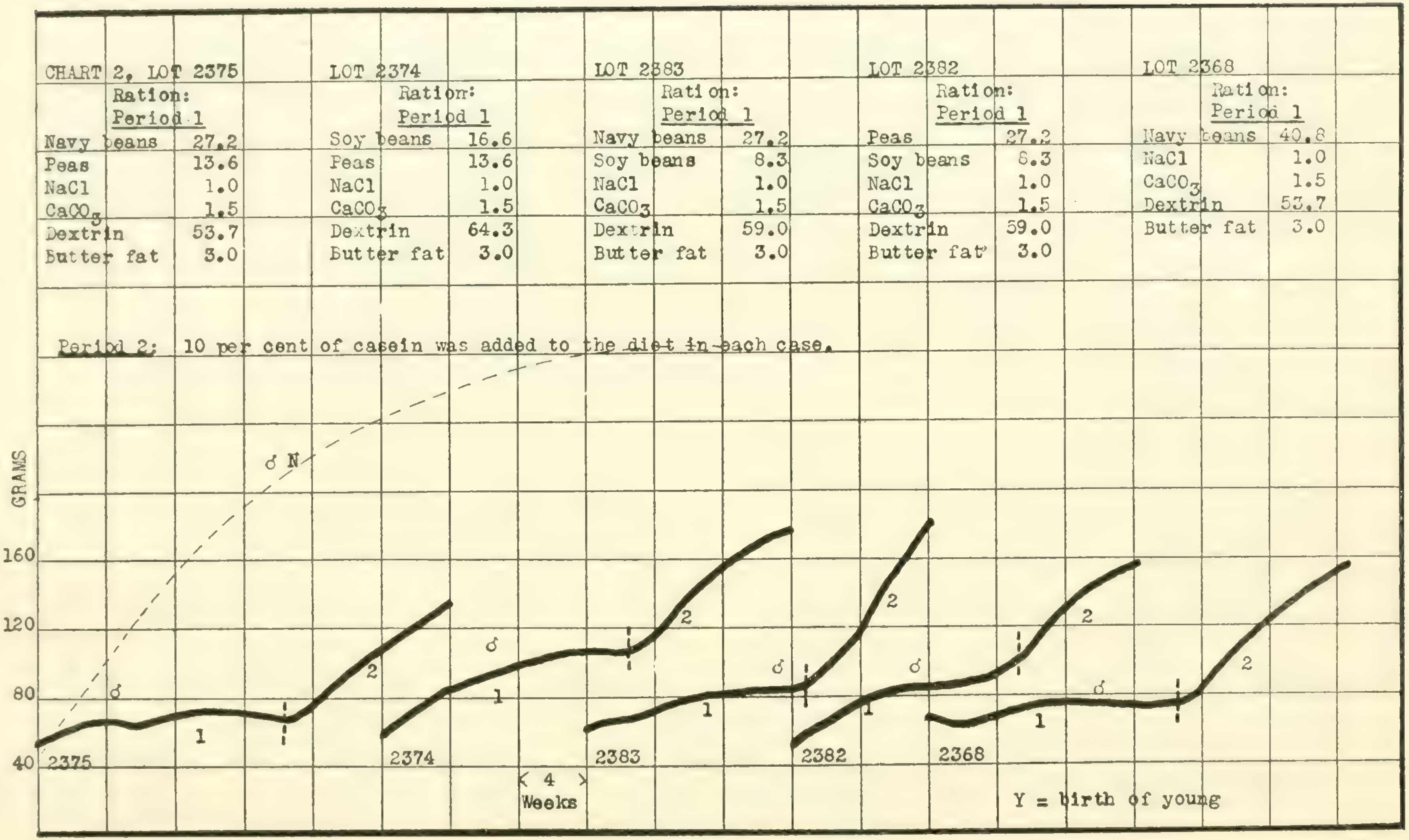

CHART 2, LOT 2375

Ration: Period 1

Navy beans	27.2
Peas	13.6
NaCl	1.0
$CaCO_3$	1.5
Dextrin	53.7
Butter fat	3.0

LOT 2374

Ration: Period 1

Soy beans	16.6
Peas	13.6
NaCl	1.0
$CaCO_3$	1.5
Dextrin	64.3
Butter fat	3.0

LOT 2383

Ration: Period 1

Navy beans	27.2
Soy beans	8.3
NaCl	1.0
$CaCO_3$	1.5
Dextrin	59.0
Butter fat	3.0

LOT 2382

Ration: Period 1

Peas	27.2
Soy beans	8.3
NaCl	1.0
$CaCO_3$	1.5
Dextrin	59.0
Butter fat	3.0

LOT 2368

Ration: Period 1

Navy beans	40.8
NaCl	1.0
$CaCO_3$	1.5
Dextrin	53.7
Butter fat	3.0

Period 2: 10 per cent of casein was added to the diet in each case.

Y = birth of young

Chart 3.—The curves in this chart illustrate the growth and reproduction records of rats fed proteins derived from two cereal grains. All factors other than protein were made satisfactory by suitable additions. In all cases the protein content of the diet was 9 per cent.

Lot 2551 derived 6 per cent of protein from rolled oats and 3 per cent from maize. Growth was only fairly satisfactory. All remained undersized. The females reached non-pregnant weights of about 150 gm. There were two females in the group but neither had any young.

Lot 2550 derived 6 per cent of protein from rolled oats and 3 per cent from wheat. These animals were undersized and not in very good condition. Three females had eleven young (two litters) but all died during the nursing period. One litter of five weighed 52 gm. at 10 days of age and was, therefore, stunted and in poor condition. At 19 days they were all dead. The stunted condition and early death of these young were probably due to the poor quality of the milk of the mother.

Lot 2547 derived 6 per cent of protein from maize and 3 per cent from wheat. These rats were undersized and their fertility was low. The growth curves of this group were not so satisfactory as we have regularly seen in animals fed a comparable diet containing 9 per cent of protein derived entirely from wheat, but were slightly better than could be secured with 9 per cent of maize protein. There were two females in this group. One remained sterile. The other had a single litter of five young, but destroyed them within a week.

Lot 2546 secured its protein entirely from wheat (6 per cent) and maize (3 per cent). While the growth curves were but slightly better in general than those of Lot 2547, the animals were in somewhat better condition.

There were two females in this group. One was sterile, while the other had but ten young (two litters) and weaned two. The nursing period was prolonged to 73 days before the young were sufficiently developed to make it possible for them to lead an independent existence on the family diet. At the age of 73 days the litter of five had been reduced to two and these weighed together but 103 gm. and were very inferior.

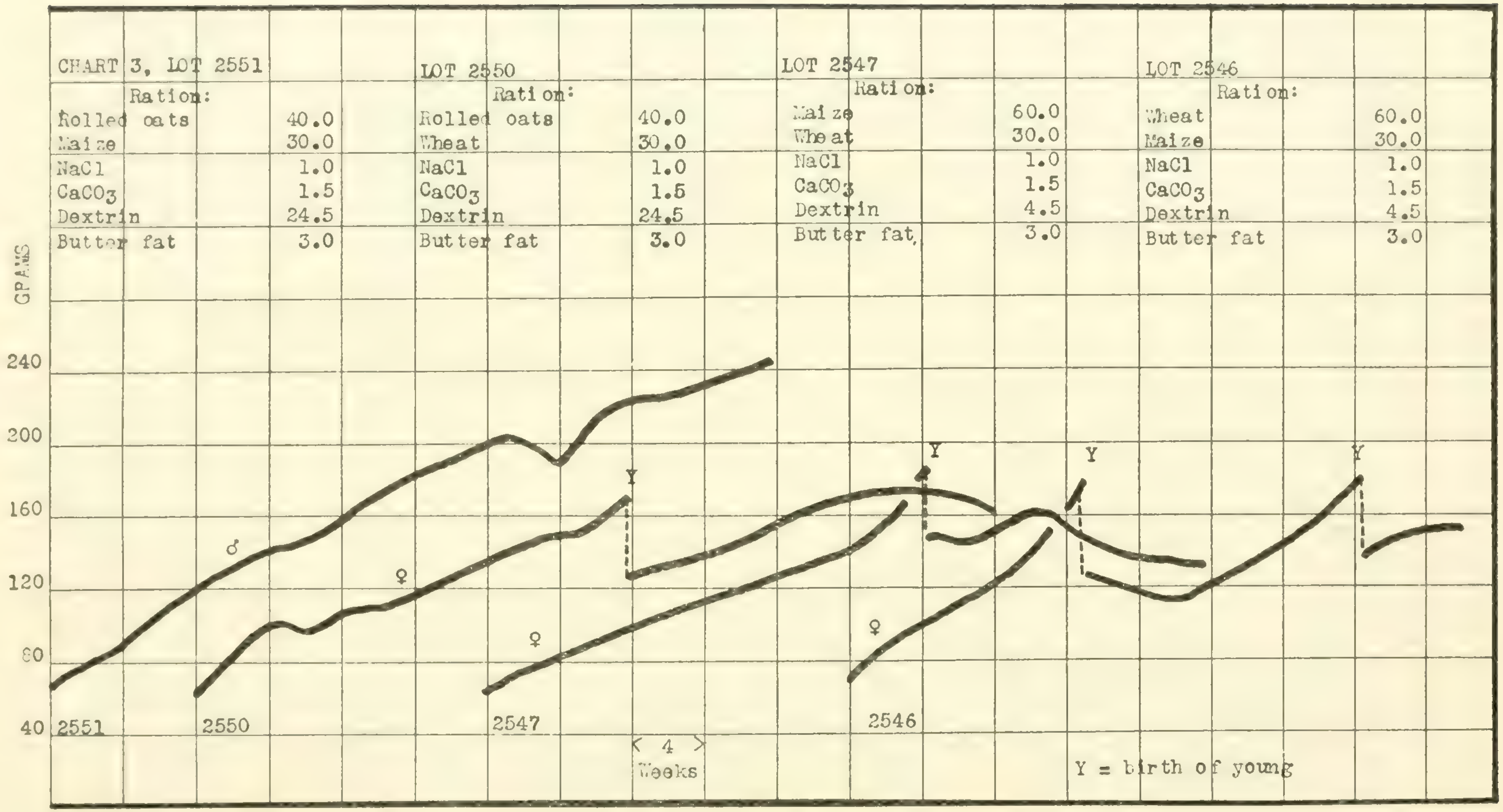
CHART 3, LOT 2551
Ration:
Rolled oats 40.0
Maize 30.0
NaCl 1.0
CaCO3 1.5
Dextrin 24.5
Butter fat 3.0
LOT 2550
Ration:
Rolled oats 40.0
Wheat 30.0
NaCl 1.0
CaCO3 1.5
Dextrin 24.5
Butter fat 3.0
LOT 2547
Ration:
Maize 60.0
Wheat 30.0
NaCl 1.0
CaCO3 1.5
Dextrin 4.5
Butter fat 3.0
LOT 2546
Ration:
Wheat 60.0
Maize 30.0
NaCl 1.0
CaCO3 1.5
Dextrin 4.5
Butter fat 3.0
GRAMS
240
200
160
120
80
40
♂
♀
♀
♀
Y
Y
Y
Y
2551
2550
2547
2546
< 4 >
Weeks
Y = birth of young

Chart 4.—Lot 2346 was fed a mixture of wheat and oat proteins in such proportions that each cereal contributed 4½ per cent of protein to the diet. These animals grew decidedly better than did those of Lot 2550 (Chart 3), which had the same protein intake but derived to the extent of two-thirds of the total from rolled oats. In oat and wheat mixtures the protein appears to be of better quality the higher the proportion of wheat.

There were two females in this group. They had together thirty-three young (seven litters) and weaned nine of them. Three second generation females remained sterile on this diet. The fertility records of this group were as much superior to those of Lot 2550 as were the growth curves. The nursing period here was again long. A litter of seven young was reduced to five by 13 days. The remaining five weighed collectively at this time 48 gm. At 32 days the five weighed 101 gm. and were in rather poor condition. At 48 days the five weighed 148 gm.

Lot 2344 was fed 9 per cent of protein derived from wheat and maize in equal proportions. The curves are better than those of Lot 2547 (Chart 3) which had 6 per cent of maize and 3 per cent of wheat proteins, respectively.

Two females had sixteen young (three litters) and weaned seven of them. These were very small for their ages. Three young at 32 days weighed 62 gm. and were poorly nourished. Two females in the second generation remained sterile.

Chart 5.—Lot 2345 derived its protein (9 per cent of the diet) from maize and rolled oats in equal proportions. This mixture was somewhat better than that of Lot 2551 (Chart 3) in which the oats and maize supplied 6 and 3 per cent of the protein, respectively.

Two females had thirty-five young (six litters) and weaned ten. Two other litters were born but their number were not ascertained because their mothers destroyed them as soon as they were born. One second generation female had fifteen young (three litters) and weaned three. The other remained sterile. The nursing period was very long. A litter of eight young was reduced by death to four by the 13th day. The remaining four weighed but 35 gm. At 62 days the three of these which remained weighed 92 gm. collectively. They were in an inferior condition.

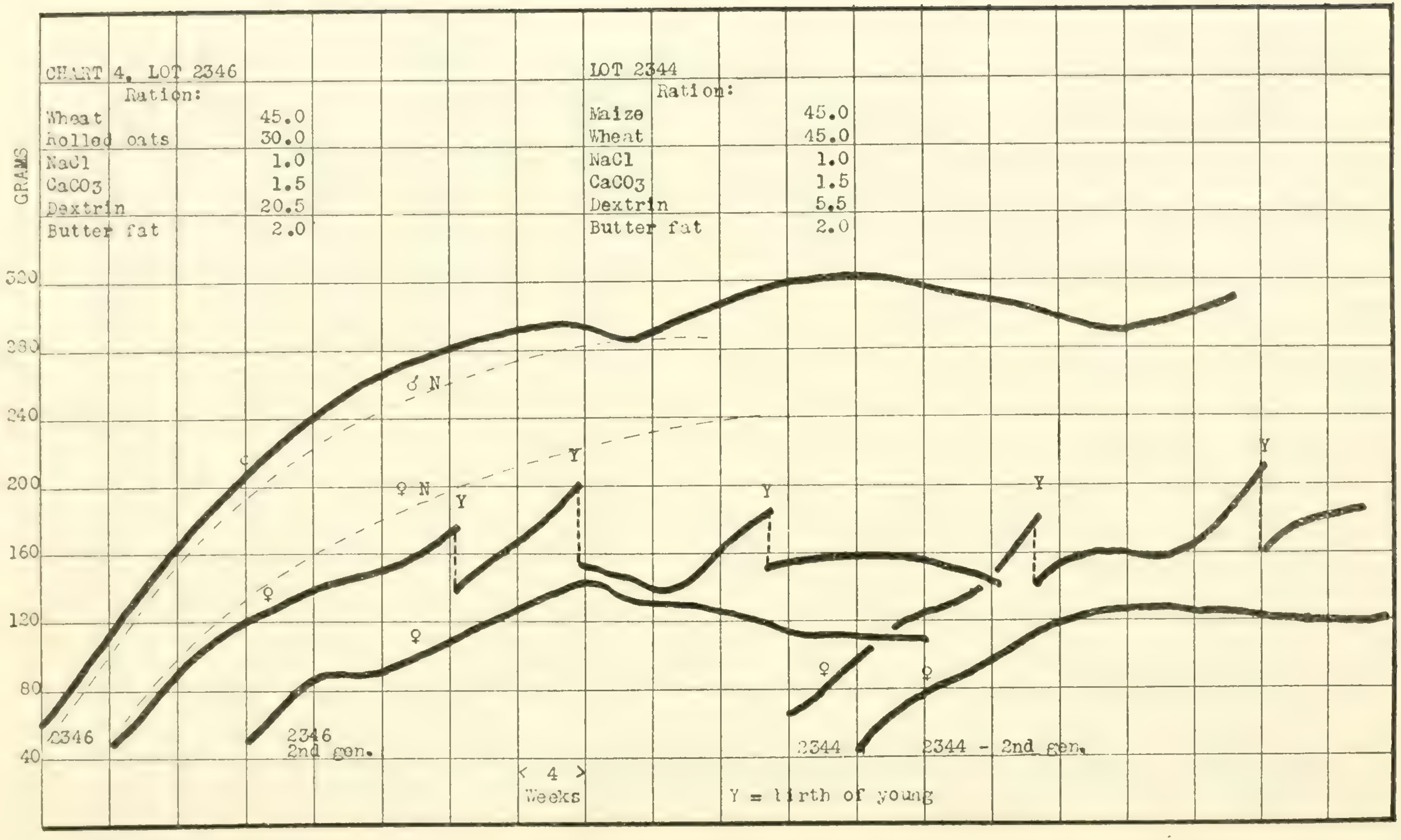

CHART 4, LOT 2346

Ration:

Wheat	45.0
Rolled oats	30.0
NaCl	1.0
$CaCO_3$	1.5
Dextrin	20.5
Butter fat	2.0

LOT 2344

Ration:

Maize	45.0
Wheat	45.0
NaCl	1.0
$CaCO_3$	1.5
Dextrin	5.5
Butter fat	2.0

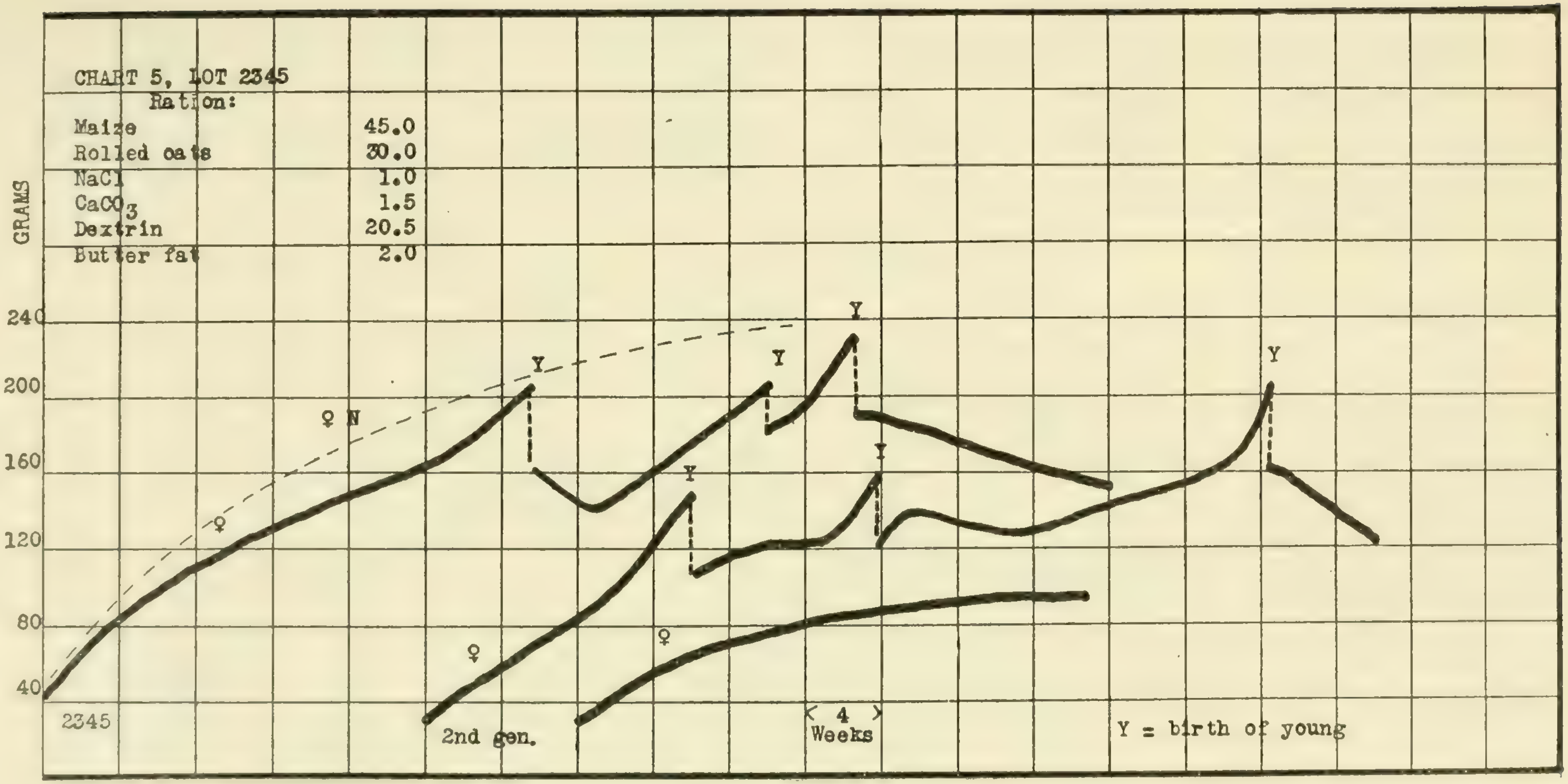
CHART 5, LOT 2345
Ration:
Maize 45.0
Rolled oats 30.0
NaCl 1.0
CaCO3 1.5
Dextrin 20.5
Butter fat 2.0
GRAMS
240
200
160
120
80
40
2345
♀ N
♀
Y
♀
2nd gen.
Y
♀
Y
Y
Y
< 4 >
Weeks
Y
Y = birth of young

Charts 1 to 5 make it clear that there is very little improvement effected in the quality of the proteins by combining two cereal grains or two legume seeds. This is so definite and striking that it forms an important contribution to the solution of the practical problems of nutrition. In Charts 6 to 13 it will be shown that in certain cases marked enhancement of the quality of the proteins is effected by making suitable combinations of a cereal grain with a legume seed.

Chart 6.—Lot 2381 derived its proteins (9 per cent of the food mixture) from barley (6 per cent) and soy beans (3 per cent). The growth curves were normal, and were therefore decidedly superior to any which we have secured with similar diets containing 9 per cent of barley proteins. 9 per cent of soy bean proteins induce but little growth when fed as the sole source of nitrogen (Chart 1, Lot 2510). These animals were still vigorous at 12 months but looked old at 17 months of age.

There were two females in the group. One was sterile, but the other had sixteen young (four litters), but did not wean any of them.

Lot 2364 was fed 9 per cent of protein derived from rolled oats (6 per cent) and navy beans (3 per cent). They remained undersized but their condition was better than that of rats fed a similar diet with but 9 per cent of oat protein as the sole source of nitrogen.

There were two females in the group. One died after 4½ months on the diet. The other had sixteen young (three litters) and weaned twelve. Six second generation females had thirty-eight young (seven litters) and weaned sixteen. Two third generation females were kept on the diet 4½ months but did not have any young. The nursing periods were rather long. A litter of five young, still with the mother, at 56 days weighed 215 gm., and were in fair condition.

Chart 7.—Lot 2378 had a diet containing 9 per cent of protein. 6 per cent was derived from wheat and 3 per cent from soy beans. The growth curves were normal, showing that there is an excellent supplementary relation between the proteins of these two seeds. This illustrates, when compared with other records of animals fed 9 per cent of proteins from various sources, many examples of which are shown in these charts, the great physiological advantage

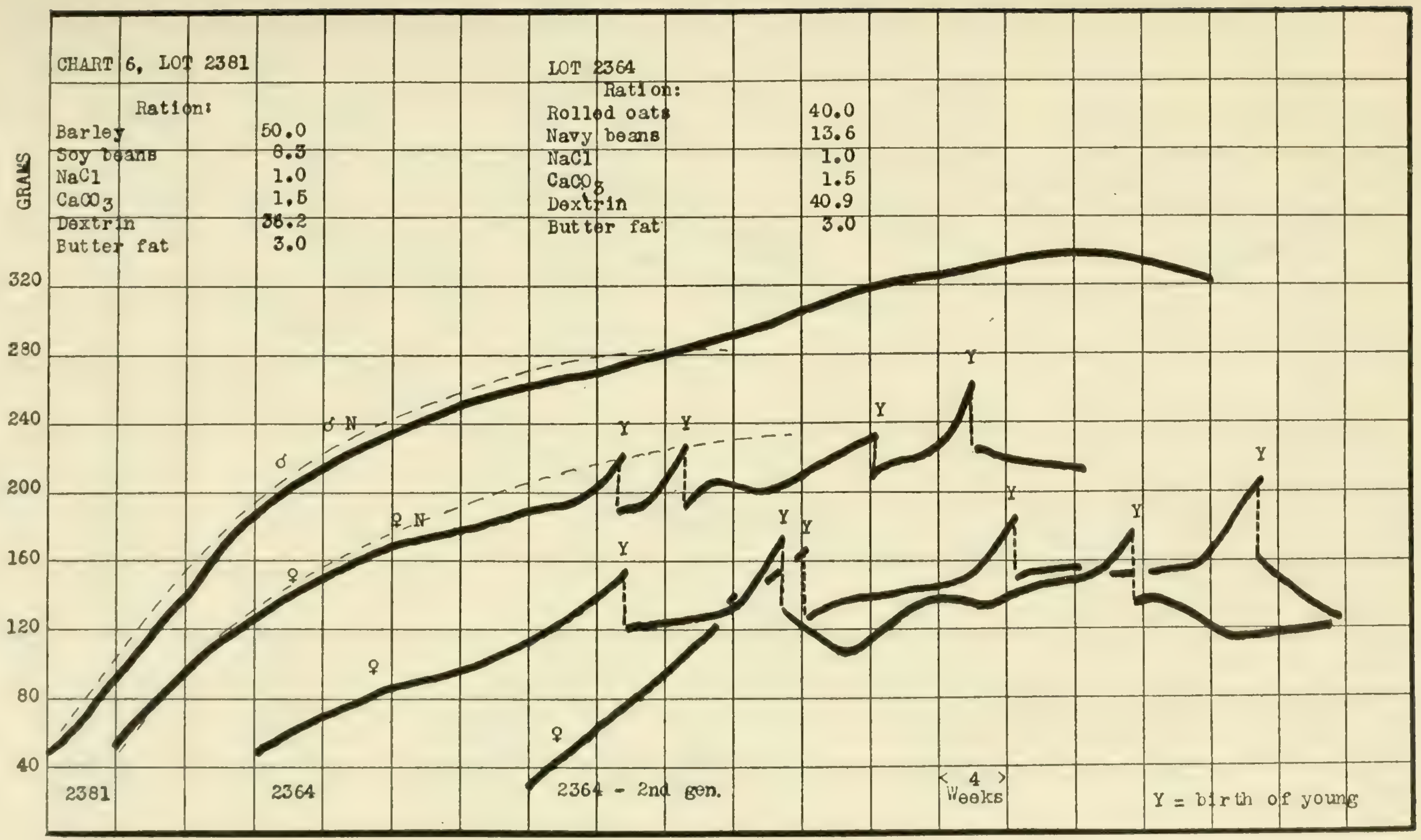
CHART 6, LOT 2381
Ration:
Barley 50.0
Soy beans 8.3
NaCl 1.0
CaCO3 1.5
Dextrin 36.2
Butter fat 3.0
LOT 2364
Ration:
Rolled oats 40.0
Navy beans 13.6
NaCl 1.0
CaCO3 1.5
Dextrin 40.9
Butter fat 3.0
GRAMS
320
280
240
200
160
120
80
40
♂ N
♂
♀ N
♀
♀
♀
Y
2381
2364
2364 - 2nd gen.
< 4 > Weeks
Y = birth of young

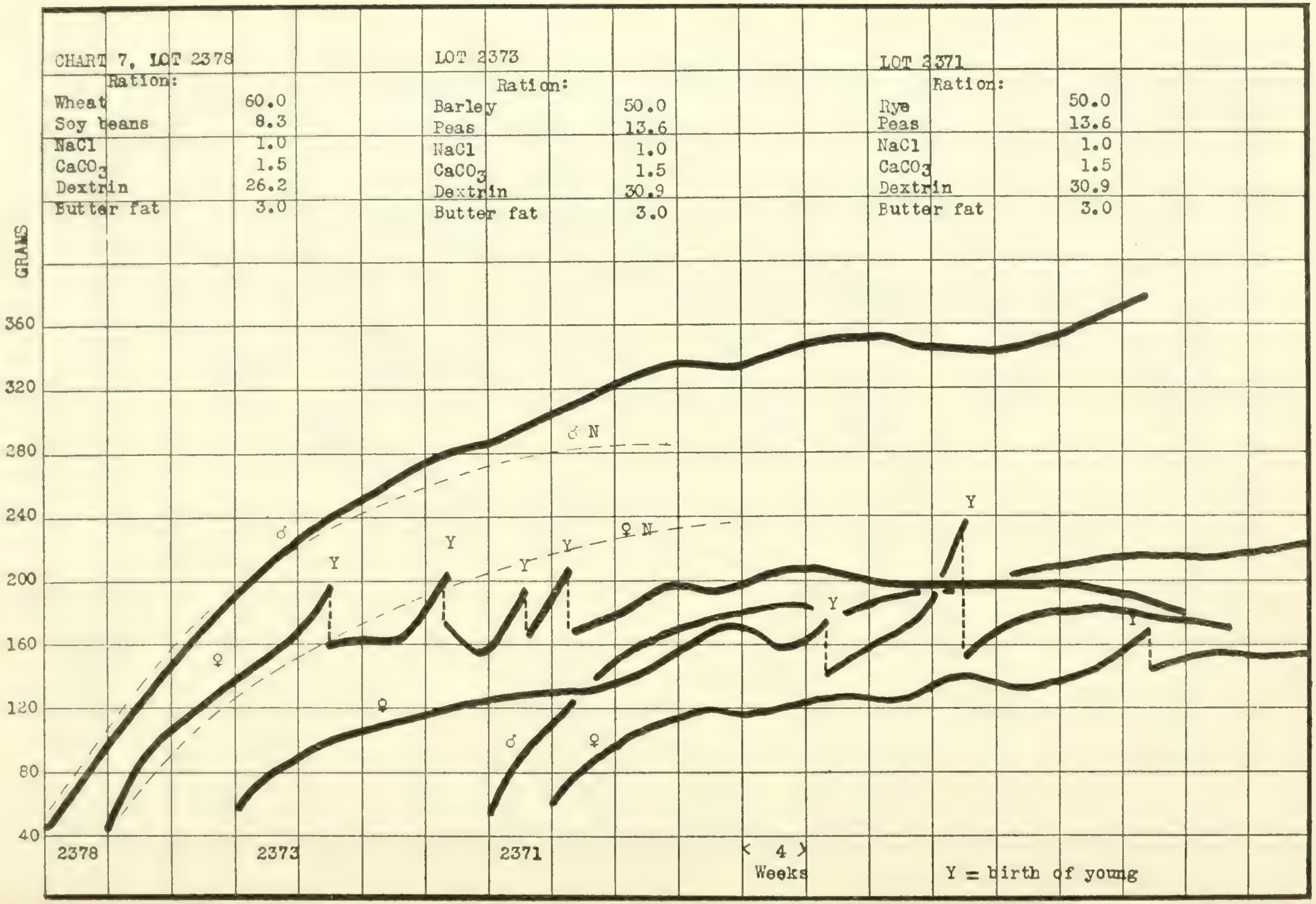
CHART 7, LOT 2378
Ration:
Wheat 60.0
Soy beans 8.3
NaCl 1.0
CaCO3 1.5
Dextrin 26.2
Butter fat 3.0
LOT 2373
Ration:
Barley 50.0
Peas 13.6
NaCl 1.0
CaCO3 1.5
Dextrin 30.9
Butter fat 3.0
LOT 2371
Ration:
Rye 50.0
Peas 13.6
NaCl 1.0
CaCO3 1.5
Dextrin 30.9
Butter fat 3.0
GRAMS
360
320
280
240
200
160
120
80
40
2378
2373
2371
♂
♀
Y
♂ N
♀ N
< 4 >
Weeks
Y = birth of young

to be derived from making appropriate combinations of natural foods so as to obtain protein mixtures having high biological values.

Two females had thirty-eight young (six litters) but destroyed them all soon after birth.

Lot 2373 derived its protein supply (9 per cent) from barley and peas. The barley supplied 6 per cent and the peas 3 per cent. These animals did not grow normally and were inferior in appearance, neglectful of their young, and were old looking at 17 months.

Three females had twenty-one young (six litters) and weaned but four. Two females of the second generation were put with the family group but were killed by the others.

Lot 2371 was fed 9 per cent of protein, 6 per cent from rye and 3 per cent from peas. The animals remained in a decidedly undersized condition and had very low fertility. One female was sterile. Another had one litter of young but destroyed them soon after birth.

Chart 8.—Lot 2365 derived its 9 per cent of protein from barley (6 per cent) and navy beans (3 per cent). The growth curves were good but the animals never reached the maximum size, nor were they in first class condition. They were very senile at 18 months of age.

Two females had thirty-three young (seven litters) and weaned eighteen, but the young were runty. They were always small for their ages and the nursing period was long. A litter of six weighed but 75 gm. when 24 days old. At 39 days five of these weighed 118 gm. At 59 days the five weighed 168 gm. This is less than one-third what they should have weighed.

Lot 2377 was fed maize equivalent to 6 per cent and soy beans equivalent to 3 per cent of the protein. The growth curves were better than can be secured with 9 per cent of maize proteins alone or with a similar amount of soy bean protein.

The females in this group were sterile. One died at 12 months, and the other at 14 months. Both were very old looking at these ages.

Chart 9.—Lot 2380 derived its protein, which constituted 9 per cent of the diet, from rolled oats (6 per cent) and soy beans (3 per cent). The growth curves do not indicate any appreciable supplementary relationship between the proteins of these two seeds.

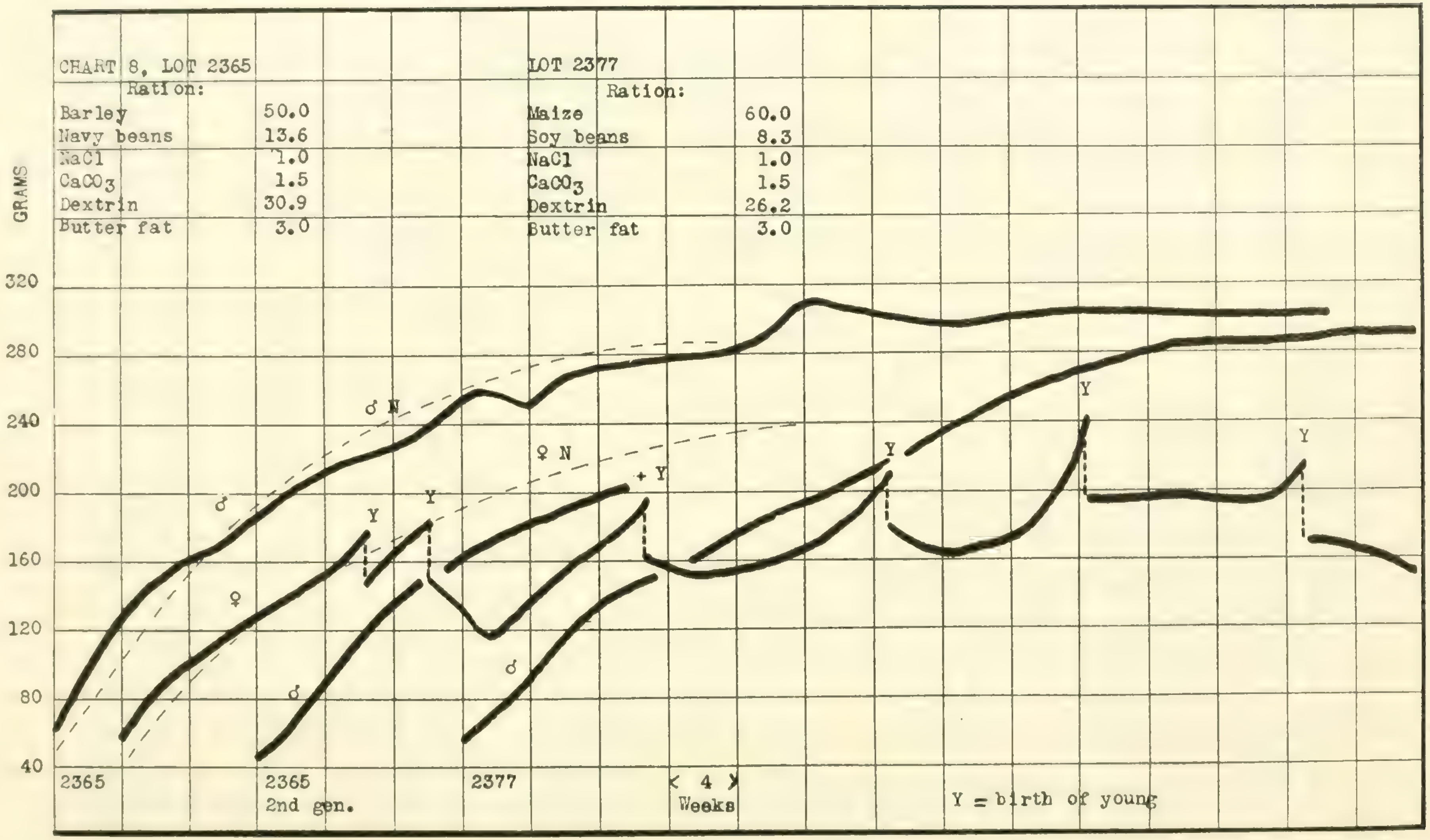
CHART 8, LOT 2365
Ration:
Barley 50.0
Navy beans 13.6
NaCl 1.0
$CaCO_3$ 1.5
Dextrin 30.9
Butter fat 3.0
LOT 2377
Ration:
Maize 60.0
Soy beans 8.3
NaCl 1.0
$CaCO_3$ 1.5
Dextrin 26.2
Butter fat 3.0
GRAMS
320
280
240
200
160
120
80
40
♂ N
♀ N
♂
♀
♂
♂
Y
Y
+ Y
Y
Y
Y
2365
2365
2nd gen.
2377
< 4 >
Weeks
Y = birth of young

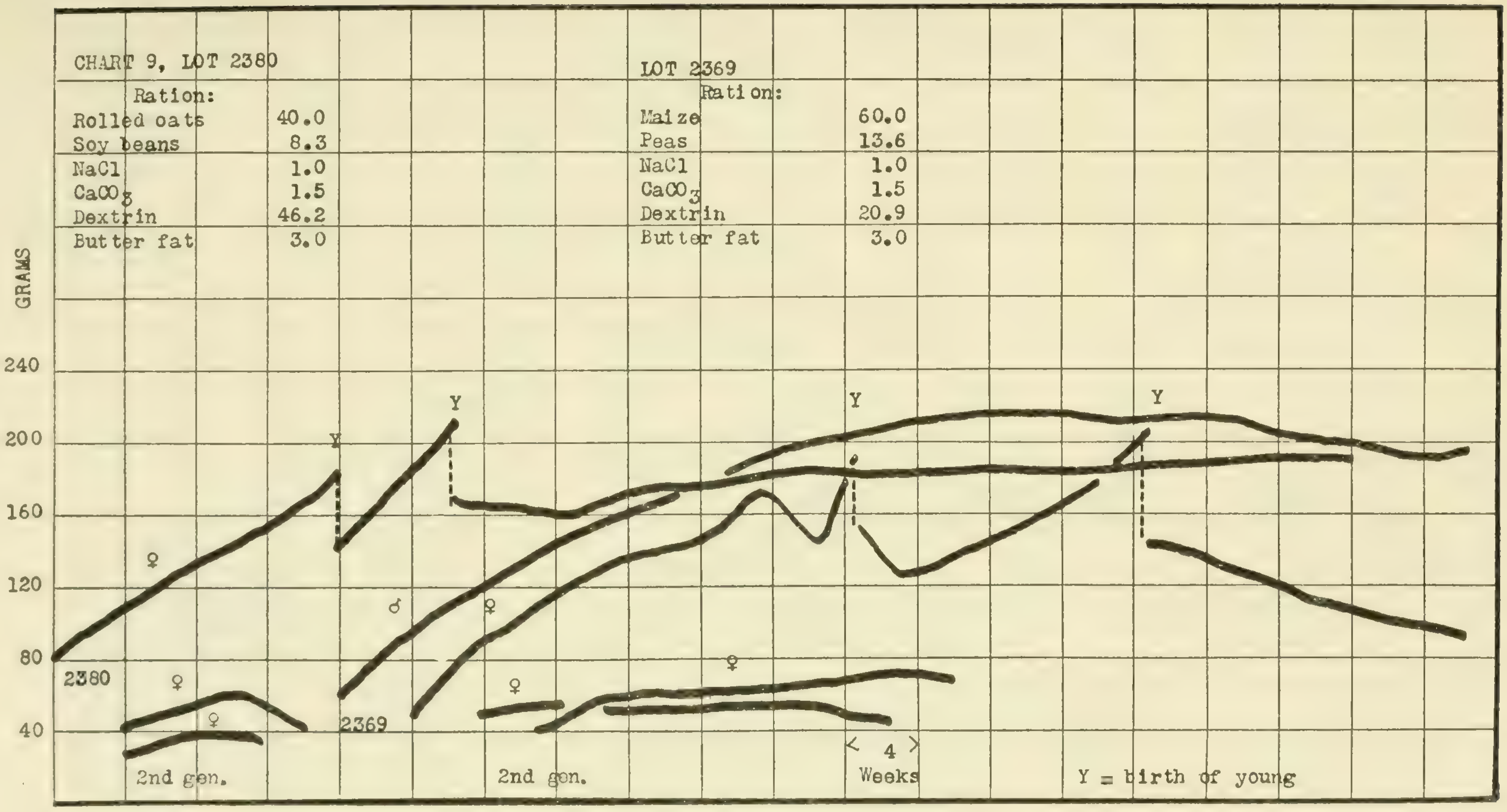
CHART 9, LOT 2380
Ration:
Rolled oats 40.0
Soy beans 8.3
NaCl 1.0
$CaCO_3$ 1.5
Dextrin 46.2
Butter fat 3.0
LOT 2369
Ration:
Maize 60.0
Peas 13.6
NaCl 1.0
$CaCO_3$ 1.5
Dextrin 20.9
Butter fat 3.0
GRAMS
240
200
160
120
80
40
2380
2369
♀
♂
Y
2nd gen.
2nd gen.
< 4 >
Weeks
Y = birth of young

Three females were confined to this diet. One never had any young. The other two had collectively fourteen young (three litters) and weaned three, which were always undersized and puny. A litter of seven was reduced by the mother to four at 16 days. At this age the four weighed 45 gm. At 35 days of age the four weighed 105 gm. and were not in a satisfactory condition. At 51 days but three survived. These weighed 102 gm. or less than half the size normal for young of this age.

Two second generation females were confined to the diet but never grew more than a few grams and died in 2 to 3 months after weaning.

Lot 2369 was fed 9 per cent of protein, two-thirds of which was furnished by maize and one-third by peas. Growth was distinctly below normal and was very inferior to a similar combination of wheat and peas (Chart 13, Lot 2370). The animals were always undersized and were old looking at 10 months and very ragged looking at 12 months.

Two females grew up on the diet. One remained sterile. The other had fourteen young (two litters) and weaned ten. The young were runty, and required a long nursing period. A litter of six young was reduced by the mother in 7 days to four. These weighed at this time 37 gm. At 31 days the four weighed 107 gm. At 46 days the four weighed 123 gm. Two females in the second generation remained undersized and never had any young.

Chart 10.—Lot 2372 derived its 9 per cent of protein from rolled oats (6 per cent) and peas (3 per cent). The growth curves were somewhat below the maximum, but much better than could be secured from 9 per cent of protein from oats or peas alone. There is, therefore, an excellent supplementary relationship between the proteins of the oat and pea, but this is not so effective as is a combination of proteins of wheat and peas (Chart 13, Lot 2370). Oat and pea proteins are better than maize and peas, as is shown by a comparison with Lot 2380 (Chart 9).

There were two females in this group. One had twenty-five young (three litters) and weaned thirteen. The young were never vigorous. One female died at 6 months from an unknown cause. One second generation female had ten young (three litters) but destroyed them soon after birth. The nursing periods were long

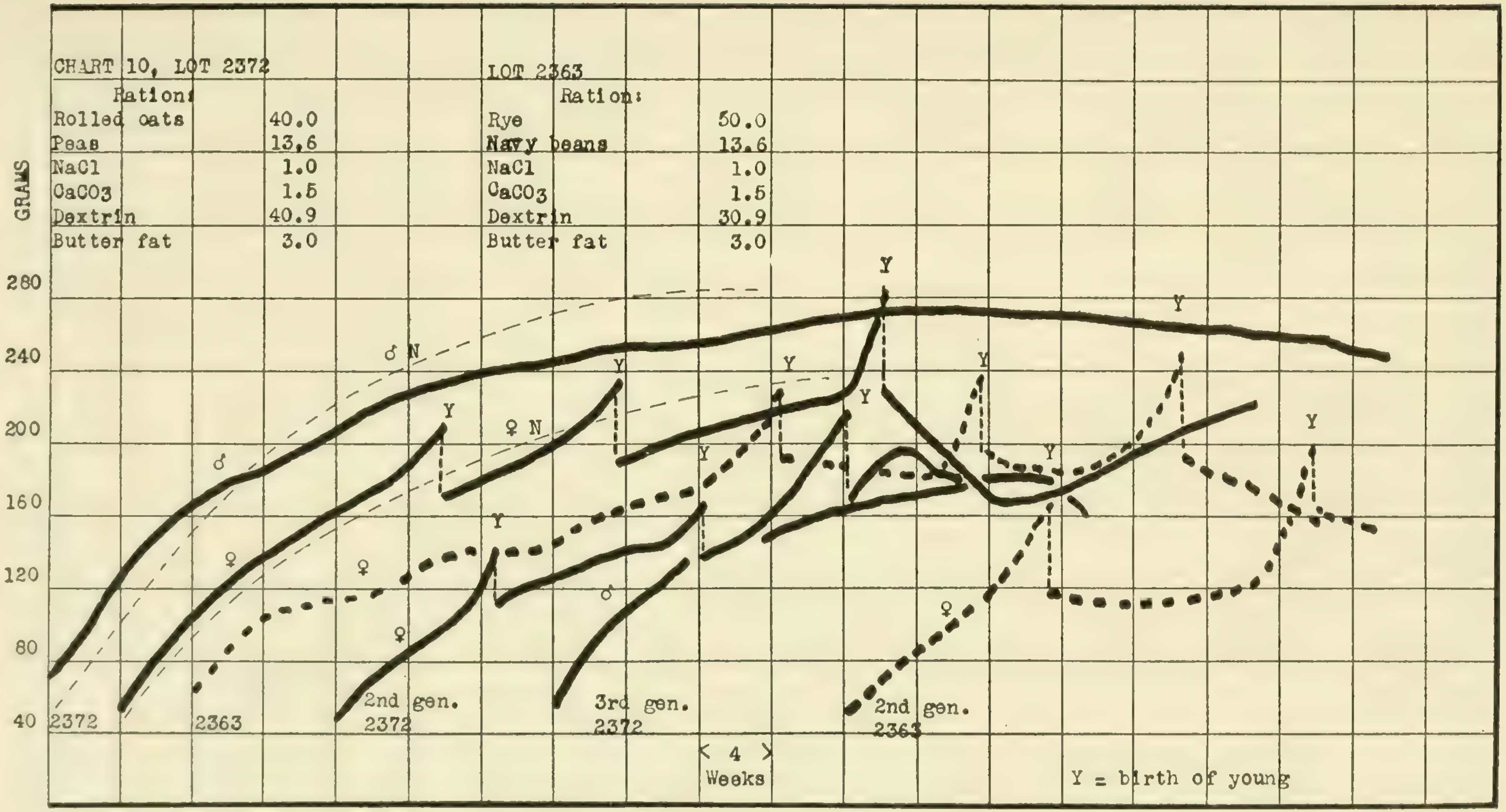
CHART 10, LOT 2372
Ration:
Rolled oats 40.0
Peas 13.6
NaCl 1.0
CaCO3 1.5
Dextrin 40.9
Butter fat 3.0
LOT 2363
Ration:
Rye 50.0
Navy beans 13.6
NaCl 1.0
CaCO3 1.5
Dextrin 30.9
Butter fat 3.0
GRAMS
280
240
200
160
120
80
40
♂ N
♀ N
♂
♀
Y
2372
2363
2nd gen. 2372
3rd gen. 2372
2nd gen. 2363
< 4 > Weeks
Y = birth of young

in all cases. A litter of eight weighed 65 gm. at 14 days. At 22 days the mother had reduced the number to six, which weighed 55 gm. At 48 days there were but five remaining. These weighed 111 gm. At 76 days but three remained. These weighed 107 gm.

Lot 2363 was fed 9 per cent of protein, 6 per cent being derived from rye and 3 per cent from navy beans. The growth records and fertility were decidedly below normal. The nursing periods were greatly prolonged and the infant mortality was high. After being restricted to the diet for 18 months the animals looked very old.

Two females had collectively thirty-one young (five litters) and weaned twenty-two. A litter of nine young was reduced by the mother to eight by the 11th day. These weighed 59 gm. They were reduced to five by 34 days, and the litter weighed 80 gm. At 55 days the litter was reduced to three, weighing 85 gm. This is less than one-third the normal weight for this age. Two second generation females had twenty-three young (four litters). One third generation female had a single litter of two, but destroyed them soon after birth.

Chart 11.—Lot 2361 was fed maize and navy beans to furnish 9 per cent of protein in the diet. Maize furnished 6 per cent and navy beans 3 per cent of the protein. The animals grew slowly and remained somewhat undersized, their fertility was low, and infant mortality high. In Lot 814 we[7] have secured much better growth and reproduction on a diet containing 7.4 per cent of maize and 4.4 per cent of navy bean proteins, respectively. This is a remarkable illustration of the physiological effects which may result from a small variation in the amount of protein in the diet.

There were two females in this group (Lot 2361). They had fourteen young (three litters) and weaned eight. The young were small for their ages. One litter of seven was reduced by the mother to six at 17 days. These weighed but 60 gm. At 42 days the six weighed 145 gm. One second generation female was kept on the diet 11½ months but never had any young.

Lot 2379 derived the 9 per cent of protein in its diet from rye (6 per cent) and soy beans (3 per cent). The animals remained somewhat undersized but appeared to be in good condition.

Two females had forty-three young (five litters) and weaned fifteen. The young were never in good condition and the nursing

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[7] McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, 1917, xxxii, 54.

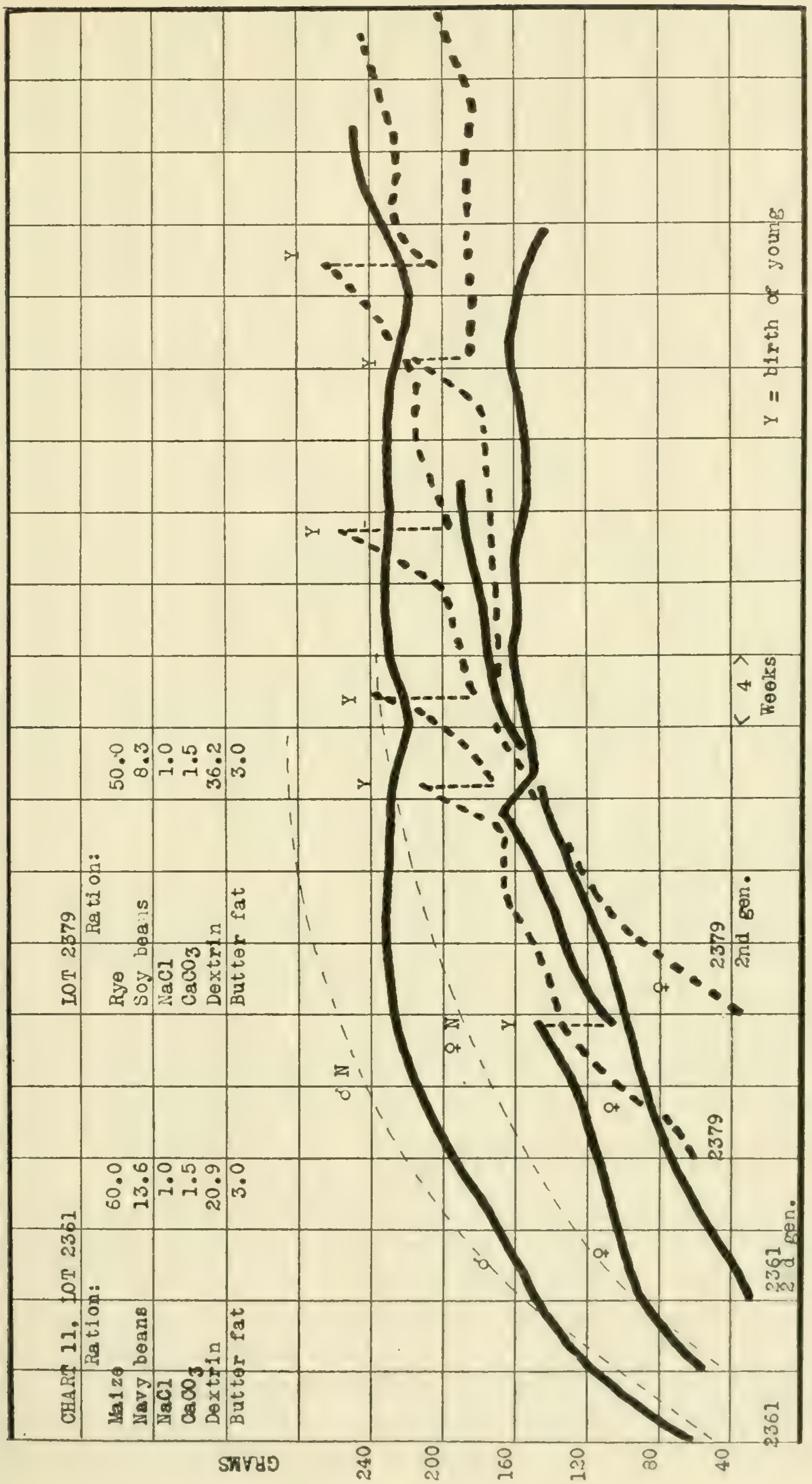
CHART 11. LOT 2361
Ration:
Maize 60.0
Navy beans 13.6
NaCl 1.0
CaCO3 1.5
Dextrin 20.9
Butter fat 3.0
LOT 2379
Ration:
Rye 50.0
Soy beans 8.3
NaCl 1.0
CaCO3 1.5
Dextrin 36.2
Butter fat 3.0
GRAMS
240
200
160
120
80
40
♂ N
♀ N
♂
♀
♀
♀
Y
Y
Y
Y
Y
Y
Y
2361
2361
2 d gen.
2379
2379
2nd gen.
< 4 >
Weeks
Y = birth of young

period was long. Three second generation females grew up on the diet. Two of these remained sterile. The other had a litter of four and weaned one.

One of the females of the original group had a litter of eleven. At 8 days these weighed collectively 85 gm. By 20 days they were reduced by the mother to ten. These weighed 110 gm. On the 35th day five of these died. On the 41st day but two were left. These weighed but 37 gm. This was less than one-third what they should have weighed. These two remaining ones were killed and eaten by the mother at 51 days. The history of this litter of young is typical of many we have seen in groups of animals restricted to diets which were satisfactory in every respect except in quality or amount of protein.

Chart 12.—Lot 2362 took a diet containing 9 per cent of protein derived from wheat (6 per cent) and navy beans (3 per cent). The growth records and fertility were good but the infant mortality was high.

Two females had thirty-seven young (nine litters) and weaned eleven. Many young were destroyed by the mothers soon after birth. Two second generation females had thirty-five young (nine litters) and weaned seventeen. One second generation female remained sterile. The young were always inferior in size, appearance, and vigor.

Chart 13.—Lot 2370 derived its 9 per cent of protein from wheat (6 per cent) and peas (3 per cent). The growth curves of this group were the best we have observed in rats fed but 9 per cent of protein derived from a combination of vegetable proteins from two sources. The animals reached full adult size, the fertility was high, and the success in rearing young was better than in any other group described in this series, or in any instance in our experience where rats were grown and maintained on a diet containing but 9 per cent of *vegetable* proteins.

Two females had forty-nine young (nine litters) and weaned thirty-four. Two second generation females were grown on the family diet. One remained sterile. The other had twenty-six young (four litters) and weaned twenty-one. One third generation female had ten young (two litters) and weaned eight. The young were always somewhat small for their ages and the nursing periods rather prolonged because of the limitation placed upon the mothers by the low protein content of the diet.

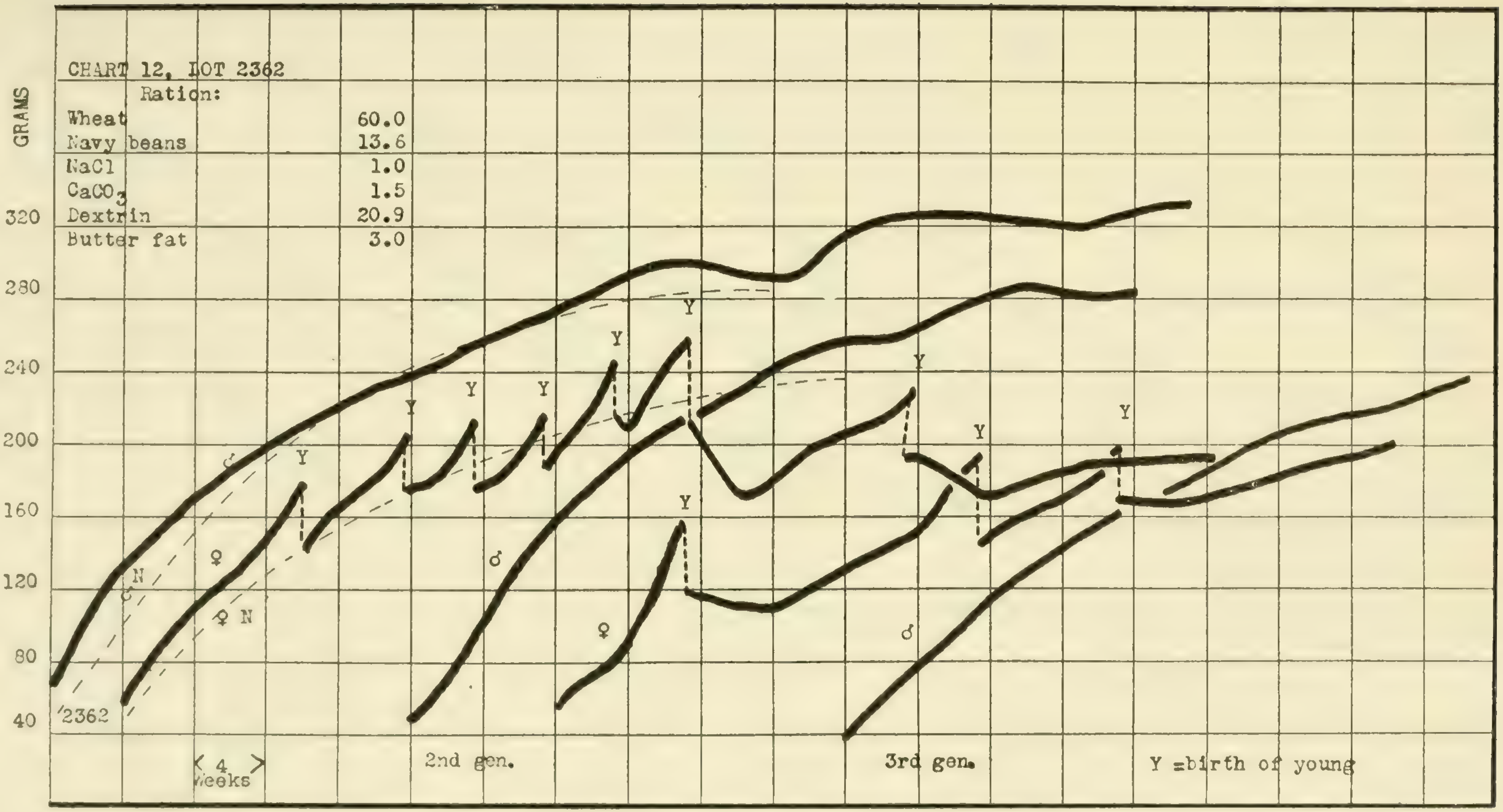
CHART 12, LOT 2362
Ration:
Wheat 60.0
Navy beans 13.6
NaCl 1.0
CaCO3 1.5
Dextrin 20.9
Butter fat 3.0
GRAMS
320
280
240
200
160
120
80
40
2362
< 4 > Weeks
2nd gen.
3rd gen.
Y =birth of young
Y
♂
♀
N
♀ N

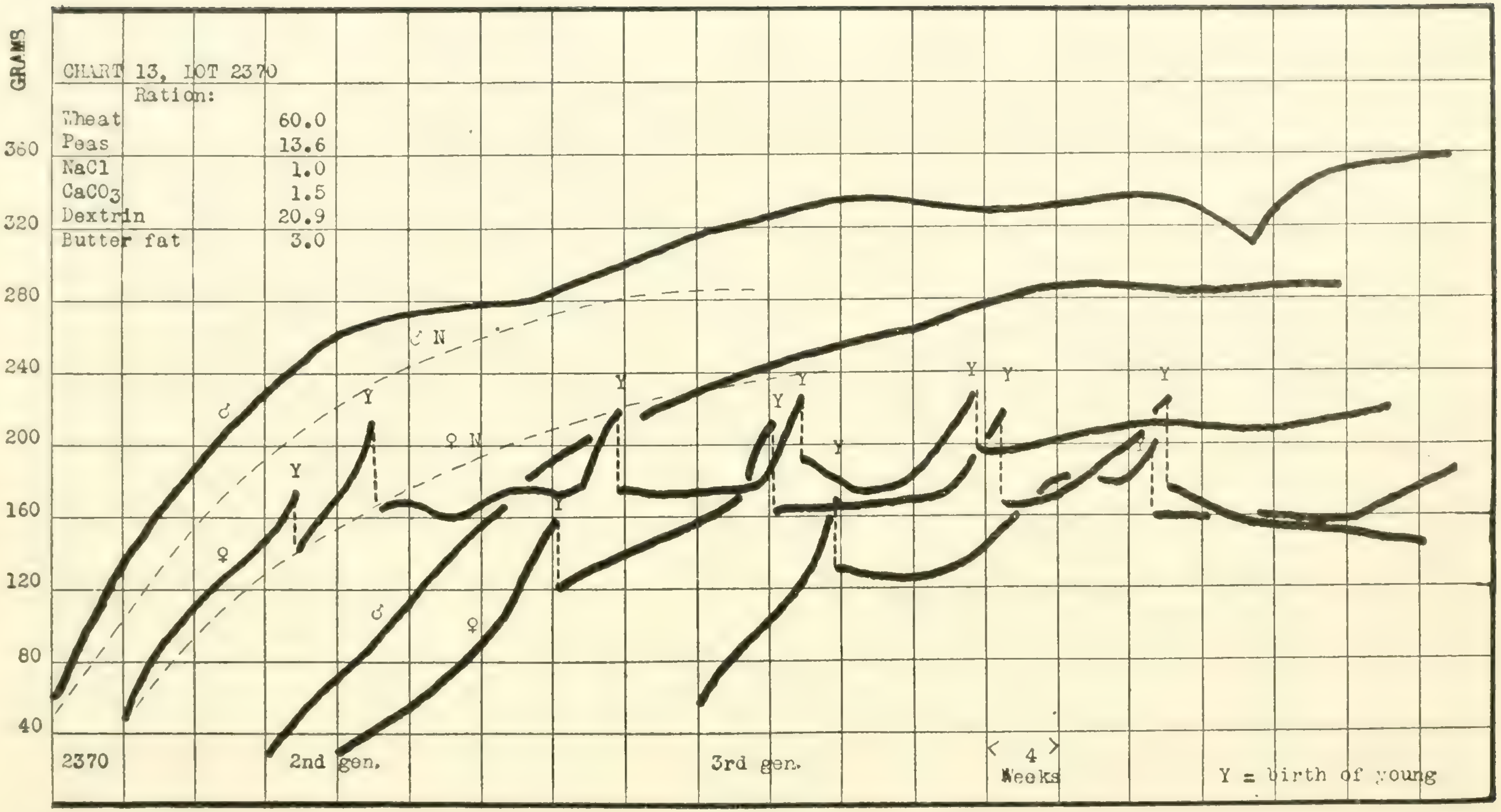

CHART 13, LOT 2370

Ration:

Wheat	60.0
Peas	13.6
NaCl	1.0
$CaCO_3$	1.5
Dextrin	20.9
Butter fat	3.0

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SUPPLEMENTARY PROTEIN VALUES IN FOODS.

V. SUPPLEMENTARY RELATIONS OF THE PROTEINS OF MILK FOR THOSE OF CEREALS AND OF MILK FOR THOSE OF LEGUME SEEDS.

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(Received for publication, March 21, 1921.)

The nature of the dietary deficiencies of the cereal and legume seeds is now well understood.[1] It has also been shown that milk and the leafy vegetables occupy a unique place among our ordinary foodstuffs in that they are the only foods regularly consumed in moderate quantities which are of a nature to correct the mineral deficiencies of cereals, legume seeds, tubers, and fleshy roots, or to adequately supplement them with respect to fat-soluble A. It is well known from laboratory experiments on animals as well as from agricultural experience, that milk proteins tend to enhance the value of vegetable proteins generally.[2] Specific information as to the extent to which milk proteins supplement those of individual vegetable foods is still wanting. The studies reported in this paper form a contribution to this phase of our knowledge of practical dietetics.

In these experiments the proteins of the diets were derived from half skimmed milk powder (Merrell-Soule) and a single plant seed or tuber. In order to bring out more clearly the extent of the supplementamirelations between the proteins employed we have in all cases liryted the content of this factor to 9 per cent of the food mixture. We have already pointed out that in order to secure a normal growth curve in the rat on this low plane of protein intake, the quality of protein for growth must be good.[3] When the growth curves fall below normal the extent of retardation of development serves as a good index to the extent to which the quality of any mixture under investigation falls below that of the best combinations which we have been able to discover. When, in addition, we observe the animals throughout their reproductive period and secure records of fertility and infant mortality, and the time at which the first signs of old age appear, we have the most sensitive indexes to physiological well being which it seems probable will ever be observable. Proteins of good quality will induce normal growth when fed at the plane of intake of 9 per cent of the food mixture, and may induce a fair degree of fertility. In order to secure high fertility and low infant mortality the proteins must be of excellent quality.

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[1] McCollum, E. V., The newer knowledge of nutrition, New York, 1918.

[2] McCollum, E. V., *J. Biol. Chem.*, 1914, xix, 323.

In a former paper[4] we have described comparable studies with diets in which the proteins were derived from combinations of either liver, kidney, or muscle with cereal or legume seed proteins. The plane of protein intake was uniform (9 per cent) in all cases. These records, together with those presented in the present paper, form, therefore, a contrast between the value of milk proteins on the one hand and animal tissue proteins on the other, as supplements for a number of vegetable foods with respect to the protein moiety.

The results bring out in a very striking way the unexpected superiority of animal tissue proteins for the special purpose of enhancing the value of various plant seed proteins. In our numerous studies of this phase of nutrition we have demonstrated that with diets of the type containing 9 per cent of protein, and with all other factors satisfactorily adjusted, the proteins of kidney produce the best results we have yet observed. The biological value of the proteins of animal tissues for growth or for maintenance of health differs in an easily demonstrable degree.

An inspection of the results of our experiments with diets of the type here employed warrants arranging the proteins of a number of animal and vegetable foods of great importance in a series showing their relative nutritive values. In the following scheme we have arranged the several foods in order of the biological value of their proteins. The best is placed at the left and the series is a descending one.

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[3] McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1919, xxxvii, 155.

[4] McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1921, xlvii, 139.

$$\text{Beef kidney—Wheat—}\left\{\begin{array}{l}\text{Milk}\\ \text{Liver—}\\ \quad\text{(beef)}\end{array}\right.\left\{\begin{array}{l}\text{Muscle}\\ \quad\text{(round steak)}\\ \text{Barley}\\ \text{Rye}\end{array}\right.—\left\{\begin{array}{l}\text{Maize}\\ \text{Oats}\end{array}\right.—\left\{\begin{array}{l}\text{Soy beans}\\ \text{Navy beans}\\ \text{Pea}\end{array}\right.$$

In our earlier studies of the cereal grains[5] we became convinced that wheat proteins were of somewhat lower biological value than more recent experimental data would seem to indicate. This may perhaps be accounted for by differences in the proteins of various samples of wheat. It is well known that the dough-forming quality varies markedly in wheats. This property depends upon the peculiar nature of the proteins of this grain and may be due to lack of uniformity in the relative amounts of the individual proteins contained in the seed.

It should be kept in mind that such a differentiation in biological value of proteins from these foods will not be apparent unless the experimental procedure is appropriate to bring them out. The several foods included in the scheme must be fed, with other dietary factors satisfactorily constituted, at such a plane of intake as to furnish the critical level of 9 per cent of protein in the diet. This is the only method we have been able to devise to show these differences. The observations must include not only the period of growth but also the fertility, the success with young, and the period following the completion of growth to the point where senile characters are apparent.

We were surprised to find how consistently combinations of milk proteins and cereal or legume proteins fail to show as high biological values as can be demonstrated for kidney, liver, and muscle proteins combined with those of certain cereals.[4] It should not be lost sight of that milk has an effective supplementary relation to cereals both with respect to the inorganic and fat-soluble A deficiencies of the latter, whereas muscle meats supplement them only with respect to the protein factor, and glandular organs only with respect to protein and fat-soluble A. This fact is brought out in a striking way by the records in Chart 5. When pasteurized or boiled milk, or cooked glandular organs are contained in the diet they do not effectively supplement a cereal and legume seed mixture with respect to the antiscorbutic factor since this is a labile substance. This factor is essential in the nutrition of man, monkey, and guinea pig, but need not be furnished by the diet of the rat or prairie dog, since they are apparently able to synthesize it.[6]

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[5] McCollum, E. V., Simmonds, N., and Pitz, W., *J. Biol. Chem.*, 1916–17, xxviii, 211. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, xxxvii, 557.

SUMMARY.

We have described in this and preceding papers experiments which were so planned as to compare the relative merits of animal tissue proteins and of milk proteins for enhancing the value of the proteins of each of the following vegetable foods: barley, peas, soy beans, rye, maize, navy beans, wheat, potato, and rolled oats. These indicate clearly that animal tissues such as liver, kidney, or muscle, are superior to milk for the specific purpose of making good the deficiencies of the proteins of the seeds and tuber mentioned above.

Milk, however, is an effective supplement for these vegetable foods with respect to other factors as well as protein. This is especially true of calcium and fat-soluble A.

In making deductions from these results it should be kept in mind that muscle tissue supplements seeds, tubers, etc., only with respect to the protein factor, and that other deficiencies of even greater importance for the well being of the body are always met with in that group of vegetable foods which are functionally storage tissues of plants; *viz.*, seeds, tubers, and roots.

Chart 1.—Lot 2391 was fed a diet containing 9 per cent of protein derived from barley (6 per cent) and milk (3 per cent). All other factors in the diet were made satisfactory by suitable additions of inorganic elements and fat-soluble A (in butter fat). Growth took place at a subnormal rate and the full adult size was never attained. The animals in this group looked rough coated and old at 15 months.

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[6] McCollum, E. V., and Parsons, H. T., *J. Biol. Chem.*, 1920, xliv, 603.

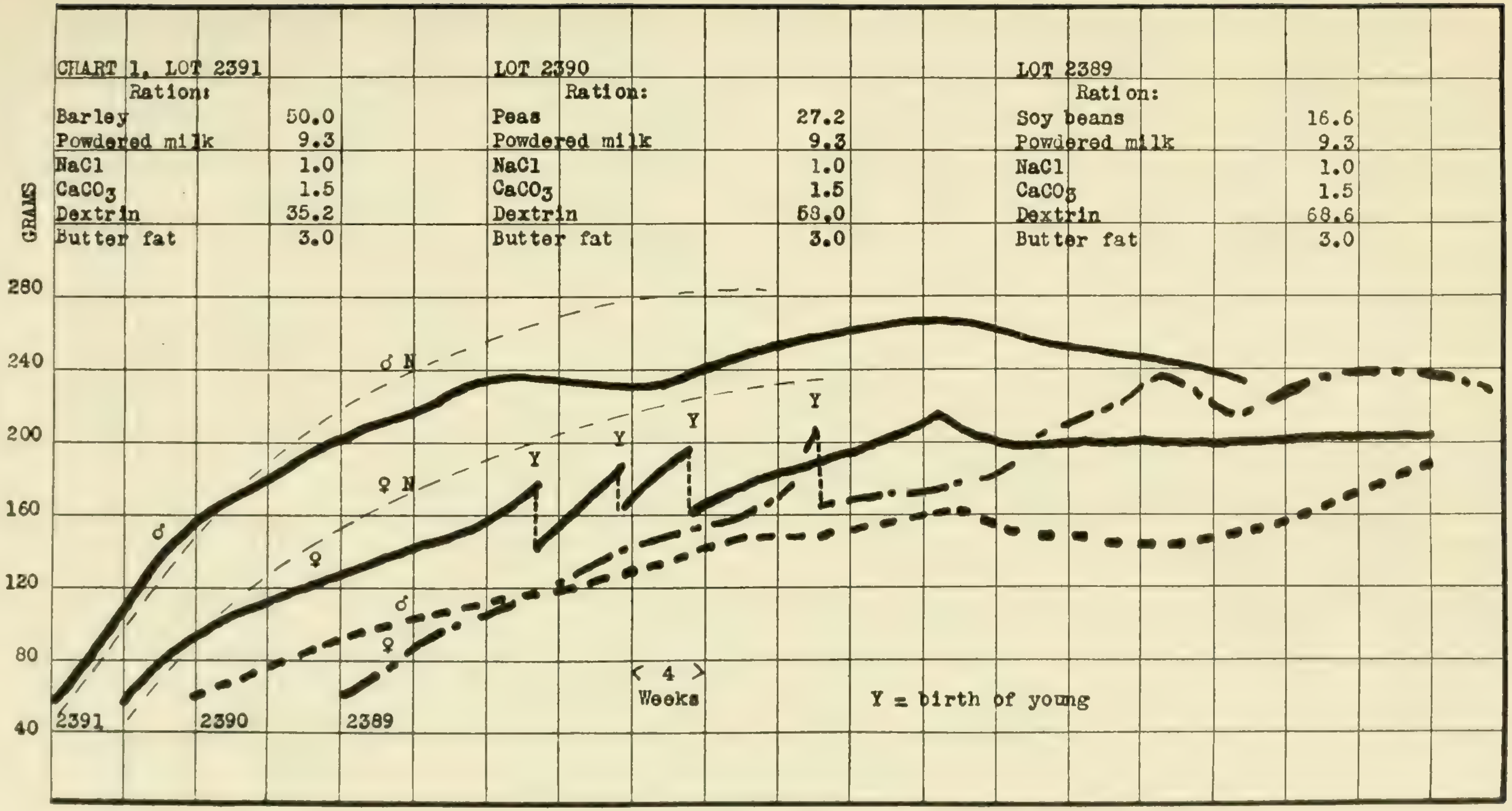


CHART 1. LOT 2391

Ration:	
Barley	50.0
Powdered milk	9.3
NaCl	1.0
$CaCO_3$	1.5
Dextrin	35.2
Butter fat	3.0

LOT 2390

Ration:	
Peas	27.2
Powdered milk	9.3
NaCl	1.0
$CaCO_3$	1.5
Dextrin	58.0
Butter fat	3.0

LOT 2389

Ration:	
Soy beans	16.6
Powdered milk	9.3
NaCl	1.0
$CaCO_3$	1.5
Dextrin	68.6
Butter fat	3.0

Y = birth of young

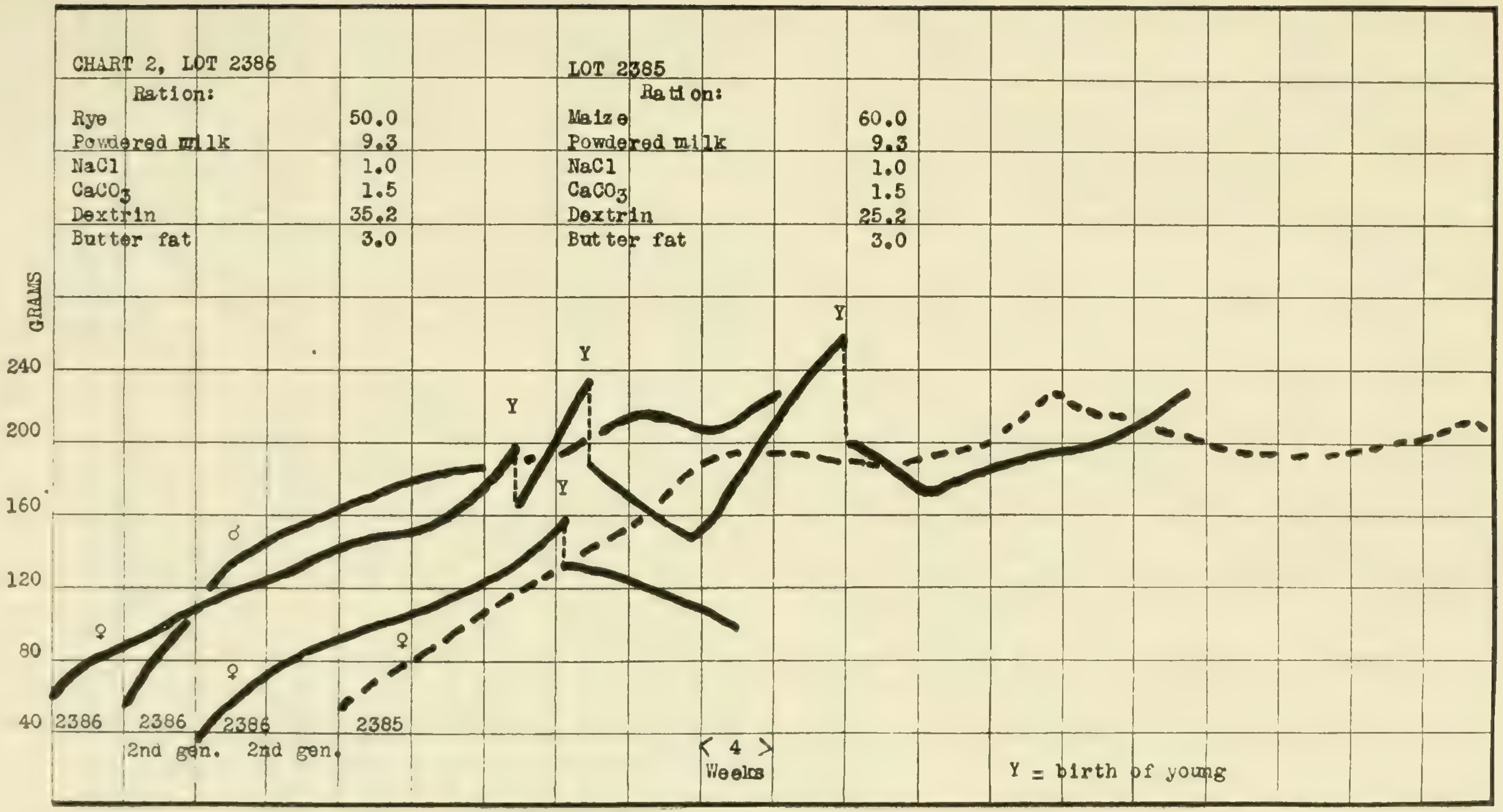
Two females grew up on this diet. One remained sterile. The other had eleven young (three litters) but destroyed them soon after birth.

Lot 2390 derived its diet, containing 9 per cent of protein, from peas (6 per cent) and powdered milk (3 per cent). The protein of the diet was the limiting factor. The animals all grew poorly, but better than they could have grown on 9 per cent of pea protein alone. These rats were very apprehensive and could be weighed only with difficulty because of constant efforts to escape. They were so excited when handled that they would not sit still in a a small covered box but would constantly spring up and strike the lid with their heads. The hair was very short and fine, and had a silky appearance, which we have never seen on rats fed highly satisfactory diets. The same type of coat has been frequently met with in rats restricted in a great measure to maize as a source of protein. Less frequently we have seen these "mole-skin" rats in groups which derived their protein from kafir corn.

Two females were restricted to this diet but never had any young.

Lot 2389 was fed soy beans and powdered milk as a source of protein. The total protein content of the diet was 9 per cent. The soy beans furnished two-thirds and the milk one-third of the total. While the growth was much better than we have ever seen on 9 per cent of soy bean protein alone, the rate of growth was distinctly below normal. The animals remained undersized, and their fertility was very low. There were three females in this group. One had a litter of seven. At 14 days they weighed 57 gm. At 21 days one had died. The remaining six weighed 62 gm. At 27 days but four survived and these weighed collectively but 45 gm. They were very puny and incapable of growing on the mother's milk. The other two females remained sterile.

Chart 2.—The rats of Lot 2386 were fed 9 per cent of protein derived from rye (6 per cent) and milk powder (3 per cent). Growth was slow but the animals reached nearly the adult size after some delay. These rats aged decidedly early. They looked old at 14 months. The second generation were all more undersized than the first. We have observed in many cases where the food mixture was faulty to a slight degree and a family was con-

CHART 2, LOT 2386
Ration:
Rye 50.0
Powdered milk 9.3
NaCl 1.0
CaCO3 1.5
Dextrin 35.2
Butter fat 3.0
LOT 2385
Ration:
Maize 60.0
Powdered milk 9.3
NaCl 1.0
CaCO3 1.5
Dextrin 25.2
Butter fat 3.0
GRAMS
240
200
160
120
80
40
♀
♂
♀
♀
Y
Y
Y
Y
2386
2386
2386
2385
2nd gen.
2nd gen.
< 4 >
Weeks
Y = birth of young

fined to the diet through several generations, each succeeding generation was smaller than the preceding one when growth was completed.

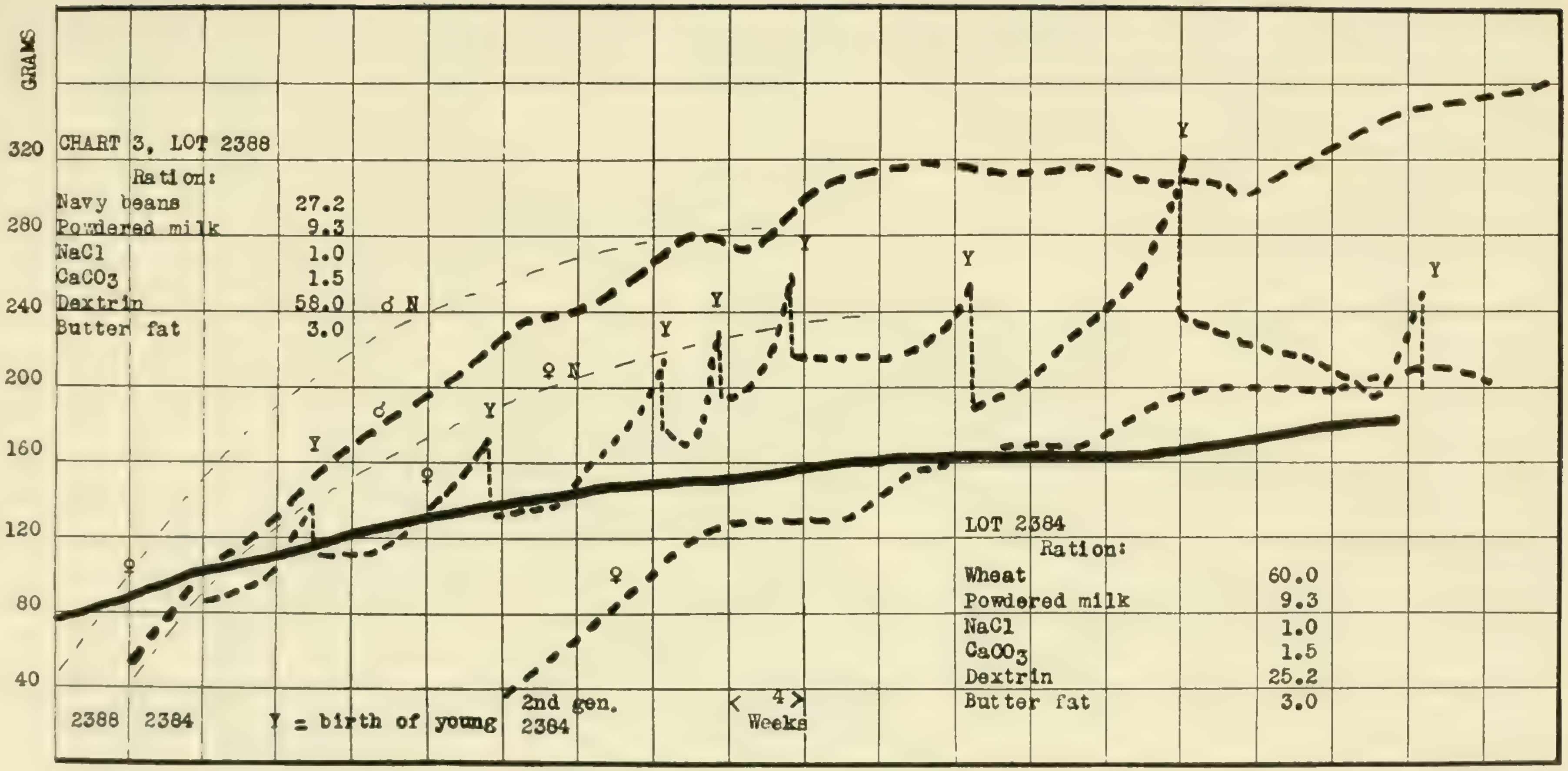
There were three females in the group. Two of these remained sterile. The other had twenty-eight young (three litters) and weaned eleven. The nursing period was, however, very long. A litter of eight was reduced by death to five by the 32nd day. These weighed 108 gm. At 39 days they weighed 121 gm., and were in a poorly developed condition. They were less than one-third the normal size for their age. One second generation female had a single litter but destroyed it soon after birth.

Lot 2385 was fed 9 per cent of protein derived from maize (6 per cent) and milk powder (3 per cent). All other factors in the diet were made satisfactory by suitable additions. Growth was slow but the animals reached nearly the full adult size. There were three females in the group but none ever had any young. The hair of this group was short and silky and suggestive of a mole skin.

Chart 3.—Lot 2388 derived the 9 per cent of protein in its diet from navy beans (6 per cent) and milk powder (3 per cent). The animals grew slowly and remained permanently undersized. They lived surprisingly long on this diet on which they grew so poorly. The history of the group on 9 per cent of protein from navy beans and milk is comparable to that of Lot 2390 (Chart 1), which was identical except that peas replaced the beans. The same diet with soy beans in place of peas or navy beans produced distinctly better growth (Lot 2389, Chart 1).

Lot 2384 derived the 9 per cent protein in its diet from wheat (6 per cent) and milk powder (3 per cent). The combination of wheat and milk proteins is better than a similar amount of protein from wheat alone. With the exception of the diet of oats and milk (Lot 2387, Chart 4), this food mixture was superior to any other combination of seed with milk proteins which we have studied. The animals appeared old after about 19 months on this diet.

There were two females in the group, one of which died after being 4½ months on the diet. The other had forty-one young (seven litters) and weaned nineteen. The nursing periods were long in all cases. The young were not destroyed in the ruthless


GRAMS
320
280
240
200
160
120
80
40
CHART 3, LOT 2388
Ration:
Navy beans 27.2
Powdered milk 9.3
NaCl 1.0
CaCO3 1.5
Dextrin 58.0
Butter fat 3.0
♂ N
♀ N
♂
♀
Y
2388
2384
Y = birth of young
2nd gen. 2384
< 4 > Weeks
LOT 2384
Ration:
Wheat 60.0
Powdered milk 9.3
NaCl 1.0
CaCO3 1.5
Dextrin 25.2
Butter fat 3.0

manner frequently observed, but died at intervals from undetermined causes.

A litter of five young weighed but 103 gm. at 23 days of age. At 58 days four weighed 141 gm. They appeared to be in good condition but were undersized. Two second generation females had but five young (one litter) and weaned one.

Chart 4.—Lot 2403 derived the 9 per cent of protein which its diet contained from potatoes (6 per cent) and milk powder (3 per cent). Growth was somewhat below normal and they remained undersized. These rats aged very early. They looked as old at 1 year as many better nourished animals do at 18 months.

Three females had twenty-seven young (five litters) and weaned only two. The nursing periods were long. A litter of five weighed 40 gm. at 15 days. At 26 days they were reduced by death to three, which weighed collectively 47 gm. At 60 days but two were left. These weighed together 70 gm. This is less than half what they should have weighed at this age.

The nitrogen of the potato is in great measure in the form of simple substances of a non-protein nature. These substances are evidently not of a character which supplements the proteins of milk to any marked extent. We have pointed out elsewhere that the nitrogen of the potato when fed as the sole source of this factor is not of so high a value as some have reported.[7]

Lot 2387 was fed 9 per cent of protein, two-thirds of which was derived from rolled oats and one-third from milk powder. This combination of proteins seems to have a higher value than any other cereal and milk mixture we have investigated. But little inferior to this is the wheat and milk combination. We have in some earlier experiments seen better curves of growth on about this amount (8 per cent) of protein from a mixture of oats and milk. After 16 months on the diet their coats (Lot 2387) were somewhat rough, but the animals were still vigorous.

Three females had thirty-three young (five litters) of which six were weaned. Three other litters were destroyed before their numbers could be determined. The nursing periods were long. A litter of seven young weighed 66 gm. at 15 days of age. At 34

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[7] McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1918, xxxvi, 197.

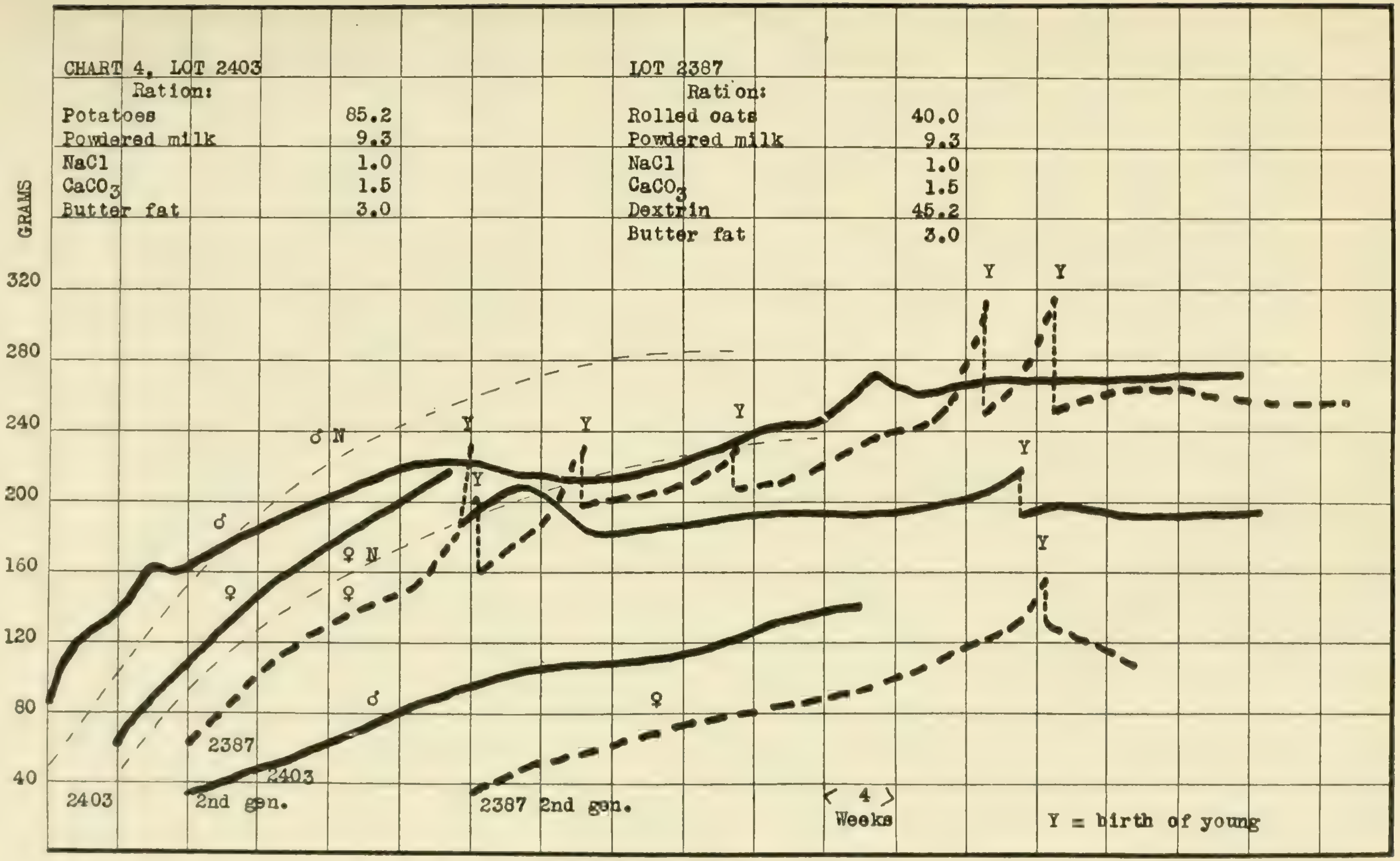
CHART 4, LOT 2403
Ration:
Potatoes 85.2
Powdered milk 9.3
NaCl 1.0
CaCO3 1.5
Butter fat 3.0
LOT 2387
Ration:
Rolled oats 40.0
Powdered milk 9.3
NaCl 1.0
CaCO3 1.5
Dextrin 45.2
Butter fat 3.0
GRAMS
320
280
240
200
160
120
80
40
♂ N
♀ N
♂
♀
♀
♂
♀
Y
Y
Y
Y
Y
Y
Y
Y
2387
2403
2403
2nd gen.
2387 2nd gen.
< 4 >
Weeks
Y = birth of young

days they weighed collectively 107 gm. At 60 days six of these weighed 202 gm., or less than half the normal for this age. Two second generation females grew up on the diet. One was sterile. The other had one litter of three after she had been 8 months on the diet. She weaned them all after a long nursing period.

Chart 5.—Lots 2148 and 2147 show the relative merits of milk and of muscle tissue as supplements to a mixture of foodstuffs consisting of articles which are functionally storage organs of plants. The mixture, aside from the milk or muscle meat, consisted of two fleshy roots, red beet and yellow turnip; a tuber, the potato; two legume seeds, pea and navy bean; and two degerminated cereal products, wheat flour and corn-meal (maize).

Notwithstanding the wide variety in such a list of foods, and an appropriate chemical composition as indicated by the ordinary food analysis, it does not promote growth in young animals nor support the vitality of adults as measured by fertility, success in rearing young, or in deferring the onset of old age.

Muscle meat (round steak) supplements a mixture of cereals, legume seeds, fleshy roots, and tubers only with respect to the protein factor. Milk on the other hand enhances not only the protein of these vegetable foods, but likewise makes good their mineral deficiencies and also the shortage of fat-soluble A which all such mixtures exhibit.

Lot 2147 in which the vegetable diet of storage organs is supplemented only with muscle meat, failed to grow normally. The curve shown is typical of a group of six animals restricted to this diet. They never had any young and aged very early. They looked extremely rough coated after 6 to 8 months on the diet. Lot 2148, on the other hand, whose diet was similar in all respects but contained 10 per cent of whole milk powder, grew normally and remained in much better condition to an age of about 18 months. These animals showed fair fertility and success in rearing their young. The milk supplemented not only the proteins of vegetable origin but accomplished what was of greater importance; *viz.*, the correction of the inorganic deficiencies and made good in great measure the lack of fat-soluble A. The bearing of such observations as these on practical human dietetics will be easily appreciated.

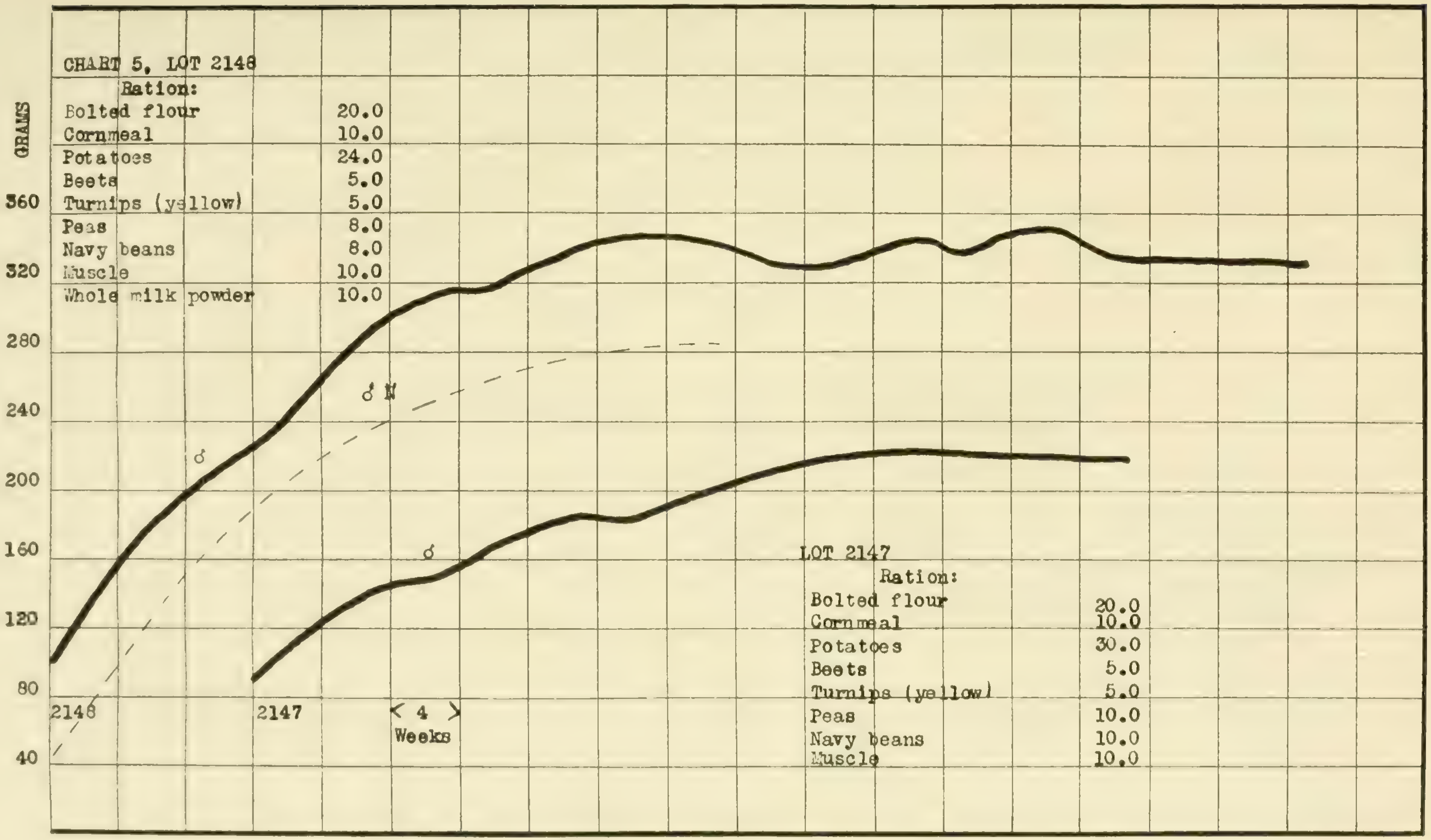
CHART 5, LOT 2148
Ration:
Bolted flour 20.0
Cornmeal 10.0
Potatoes 24.0
Beets 5.0
Turnips (yellow) 5.0
Peas 8.0
Navy beans 8.0
Muscle 10.0
Whole milk powder 10.0
GRAMS
360
320
280
240
200
160
120
80
40
2148
2147
< 4 >
Weeks
♂
♂ N
♂
LOT 2147
Ration:
Bolted flour 20.0
Cornmeal 10.0
Potatoes 30.0
Beets 5.0
Turnips (yellow) 5.0
Peas 10.0
Navy beans 10.0
Muscle 10.0

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THE CULTIVATION OF YEAST IN SOLUTIONS OF PURIFIED NUTRIENTS.

By MARGARET B. MacDONALD and E. V. McCOLLUM.

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(Received for publication, November 16, 1920.)

There has existed since the controversy between Pasteur and von Liebig (1, 2, 3) and the subsequent work of Mayer (4) and von Nägeli (5) a belief that yeast in nutrient solutions affording only ammonium salts as a source of nitrogen failed to multiply to an appreciable extent. The addition of small quantities of impurities of certain kinds, especially yeast extract, exerted a profound effect on the growth of yeast cells in such media. The nature of the substance or substances which affect the multiplication of yeast cells in so marked a degree has never been clearly stated. To define it Wildiers (6) coined the term "bios." Yeast itself was considered the best source of this growth-promoting substance. Very small amounts of "bios" were needed to insure rapid proliferation, and it was thought that the better growth secured in nutrient solutions of purified foodstuffs in which an ammonium salt served as the sole source of nitrogen when a large seeding of yeast was made, as contrasted with the results when one or very few cells were cultivated, was due to the death and autolysis of some of the cells. These dead cells contributed the hypothetical "bios" to the solution, and thus enabled the living ones to proliferate.

The proofs brought forward in support of this view have not been entirely convincing, but have been widely accepted. That the acceptance has not been universal is made evident by the numerous papers that are still being written in support of or in opposition to it. Quite recently it has been suggested that the "bios" of Wildiers is perhaps identical with the antineuritic substance concerned in the nutrition of the higher animals. Williams (7) and Bachmann (8) have devised procedures for the detection and approximate estimation of the antineuritic substance by the use of yeast as a test organism. Their methods are based on the assumption that there exists a definite requirement by yeast of the antineuritic substance which has generally been assumed to be identical with the dietary factor water-soluble B. These authors hold the view that they have demonstrated experimentally that the multiplication of yeast cells in a solution containing pure chemical substances, all of which can be named, and the fermentative power of the yeast are quantitatively influenced by the addition of substances known to contain water-soluble B (7, 8). In a recent paper from this laboratory (9) it has been shown that extracts of certain natural foods, *e.g.* wheat germ, rolled oats, muscle tissue, etc., even when treated so as to contain so little of the antineuritic substance that its presence cannot be demonstrated experimentally with rats, still exert a profound influence on the proliferation of yeast cells. The same is true of the addition of mixtures of amino-acids and of glucose. The view that the growth of yeast can be made a quantitative method for the dietary factor water-soluble B must be regarded as questionable until supported by more convincing experimental proof.

The following experimental work was undertaken in order to throw further light on the problem of the requirement of yeast of the dietary factor water-soluble B. The problem might also be stated, because of the nature of the experimental work and the line of reasoning involved, to be a test of the identity of the antineuritic substance water-soluble B with "bios."

We were successful in securing the multiplication of yeast in fifteen successive seedings in nutrient solutions of purified chemical substances.

In order to have a synthetic solution free from any trace of water-soluble B it seemed expedient to restrict the nutrient substances as nearly as possible to those of mineral origin. More than half a century of experimentation on the nutrition of yeast furnished abundant material for references. According to Lafar (10) yeast may under proper conditions make excellent use of ammoniacal nitrogen. Enough experimental evidence in favor of at least a partial utilization of an inorganic nitrogen supply by yeast cells was found to warrant the use of ammonium sulfate as the source of nitrogen. The sucrose used as the carbohydrate supply was prepared from the best granulated cane sugar by repeated precipitation from a concentrated water solution by absolute alcohol.

Nutrient Solution 1. 2 Per Cent Sucrose.

1 liter distilled water.
20 gm. sucrose, recrystallized.
3 " ammonium sulfate, C.P.
2 " potassium dihydrogen phosphate, C.P.
0.25 gm. calcium chloride, C.P.
0.25 " magnesium sulfate, C.P.

Nutrient Solution 2. 5 Per Cent Sucrose.

The second nutrient solution differed from the first only in that it contained 50 gm. of sucrose per liter.

The solutions were heated to boiling on 2 successive days. On the 3rd day portions of 25 cc. were measured with a sterile pipette into a number of small sterile Erlenmeyer flasks, which were then heated to boiling, stored in a cupboard, and kept free from possibilities of contamination.

Three strains of yeast, baker's yeasts "F" and "XII" and brewer's yeast "K," were obtained from the Fleishmann Laboratories. A small loopful of these pure cultures was used to seed 25 cc. of sterile nutrient solution. The flasks were then well shaken to distribute the cells and allowed to stand undisturbed in the cupboard at room temperature. There was not enough yeast to produce visible turbidity in the solutions. At the end of a week there was a very perceptible growth of yeast on the bottom of the flasks and the solutions showed considerble turbidity when shaken. 1 cc. of the well agitated suspension in Flask A was transferred to a second seeding, Flask B, which was kept for a week, when the turbidity seemed about the same as in Flask A 1 week after seeding. A third seeding was made from Flask B to Flask C. This successive seeding was continued to the fifteenth seeding with no apparent change in the rate of growth or diminution in the fermentative activity of the yeast. Every precaution was taken to insure pure seedings, a microscopic examination of a sample from each flask being made before seeding the next flask, because of the known influence of other fungi (11, 12).

The seedings in Nutrient Solution 2 were conducted in the same way as those in Nutrient Solution 1. It was, however, possible to make seedings every 5 days rather than every 7 days as in the first series. It was noted in both series that the two strains of baker's yeast, "F" and "XII," made a somewhat better growth than brewer's yeast "K."

Flask A containing 25 cc. of nutrient solution had added to it the "bios" contained in one loopful of a pure culture of yeast cells. If these cells gained no "bios" and lost none, Flask B received 1/25, Flask C received 1/25 of 1/25, or 1/650, of the original amount of "bios," and Flask J received 1/5, 667,253,614,400; *i.e.*, less than one-five trillionth of the "bios" in the one loop of yeast cells used to seed Flask A. If, as Amand (12) supposes, the original amount of "bios" decreases very rapidly, it must appear that yeast may grow without "bios" or water-soluble B, or it must be able to synthesize its own supply.

The fermentative power of the yeast used in these experiments was determined for cells taken from the fifteenth seeding. 0.5 cc. of yeast suspension was used for each test. The examination was carried out according to Durham's fermentation test with glucose solution.

	Gas. *inches*
Yeast XII after 3 days	$3\frac{1}{2}$
" K " 3 "	$3\frac{3}{4}$
" F " 3 "	$3\frac{1}{2}$
Control " 3 "	0

There can be no question about the remarkably stimulating effect of adding various substances such as amino-acids and other extractives to such purified nutrient solutions as were used in this work. Where extracts of natural foods are employed, using yeast as a test organism with a view to determining by the extent of the proliferation of the cells the content of antineuritic substance in such extracts, there is always added with the antineuritic substance so many others having a favorable influence on the growth of yeast that it seems unlikely that the test as carried out by Williams and Bachmann contains any element of specificity.

SUMMARY.

The results of our efforts to cultivate yeast in nutrient solutions containing no possible source of the antineuritic factor have been of such a nature as to make us seriously question whether yeast is dependent on a supply of the antineuritic principle for its continued multiplication. Successive seedings with very few cells have been carried sufficiently far to reduce the possible content of antineuritic substance ("bios," "water-soluble B") to vanishingly small amounts.

We have grown quantities of yeast in this purified nutrient solution sufficient to furnish from 2 to 5 gm. of dry substance. It would seem that but one of two conclusions is admissible; *i.e.*, either yeast must grow without "bios" or it must synthesize the substance to meet its own needs (as was believed by Henry (13)). Feeding experiments with yeast grown under the conditions described in this paper are contemplated and should yield results of unusual interest.

BIBLIOGRAPHY.

1. Pasteur, *Compt. rend. Acad.*, 1858, xlvii, 1011.
2. von Liebig, J., *Ann. Chem.*, 1870, cliii, 1, 137.
3. Pasteur, L., *Ann. chim. phys.*, 1872, xxv, 145.
4. Mayer, A., *Ann. Physik*, 1871, cxlii, series 1, 293.
5. von Nägeli, C., *Chem. Zentr.*, 1881, xii, 45.
6. Wildiers, E., *La Cellule*, 1901, xviii, 313.
7. Williams, R. J., *J. Biol. Chem.*, 1919, xxxviii, 465; 1920, xlii, 259.
8. Bachmann, F., *J. Biol. Chem.*, 1919, xxxix, 235.
9. Souza, G. de P., and McCollum, E. V., *J. Biol. Chem.*, 1920, xliv, 113.
10. Lafar, F., Handbuch der Technischen Mykologie, Jena, 1905–08, 155.
11. Kossowicz, A., *Chem. Zentr.*, 1903, lxxiv, pt. i, 475.
12. Amand, A., *La Cellule*, 1904, xxi, 329.
13. Henry, J., *Ann. brass. et dist.*, 1902, 129; *Woch. Brau.*, 1902. xix, 325.

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THE "BIOS" OF WILDIERS AND THE CULTIVATION OF YEAST.

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(Received for publication, March 30, 1921.)

Through the courtesy of the Editors of the Journal, Prof. Ide's paper was submitted to us before publication. We are taking this opportunity to comment on the several points which he has raised.

The object of our study was to convince ourselves whether yeast could be utilized as a test organism for the approximate estimation of the content of the antineuritic substance (water-soluble B) in foodstuffs or preparations made from them. The investigation had its beginning in the efforts of Souza[1] to apply the methods described by Williams[2] and by Bachmann.[3] Souza was unable to obtain sufficiently uniform results to inspire confidence that the method had any value for the specific purpose for which it was designed.

Following this experience an investigation was made of the effects of extracts of wheat germ, beefsteak, and rolled oats, respectively, prepared after these materials had been heated with alkali sufficiently long to destroy all the antineuritic substance which they contained, or at least a sufficient amount to make negative efforts to detect it by administering the preparations to polyneuritic rats. Extracts of these alkalinized foods were found to stimulate the growth of yeast when added to our standard nutrient solution of inorganic substances and sugar, in a manner entirely comparable with similar amounts of extracts prepared from untreated wheat germ, muscle, or oats. Since these extracts were entirely without curative properties when administered to polyneuritic rats, there was no parallelism between the antineuritic factor and the development of yeast, and we were forced to the conclusion that some factor other than the antineuritic substance was responsible for the stimulation of the yeast growth.

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[1] Souza, G. deP., and McCollum, E. V., *J. Biol. Chem.*, 1920, xliv, 113.

[2] Williams, R. J., *J. Biol. Chem.*, 1919, xxxviii, 465.

[3] Bachmann, F. M., *J. Biol. Chem.*, 1919, xxxix, 235.

To supplement Souza's investigation, we undertook to grow pure cultures of three races of yeast in the nutrient medium containing only ammonium sulfate as a source of nitrogen, the other essential inorganic nutrients and sugar being purified by repeated precipitation from concentrated aqueous solution by means of alcohol. This nutrient solution was, we believed, far more carefully prepared so as to insure freedom from even traces of the antineuritic substance or from other unknown complexes, such as the hypothetical "bios," than any medium employed by previous investigators. Since these pure races of yeast grew just as well at the end of a series of 15 consecutive seedings in this nutrient solution as they did earlier, we were forced to the conclusion that yeast can grow without either the antineuritic substance or the hypothetical "bios."

We are fully in accord with Prof. Ide in his statement that there is a most striking difference in the rate of proliferation of yeast in the purified nutrient solution, as contrasted with the same after the addition of extracts of natural foods. The explanation for this may probably be considered of twofold character. In an attempt to explain this, Souza and McCollum inclined to the view that the added extracts were potent because of improvement in the nutrient substances. The recent publication of Fulmer, Nelson, and Sherwood[4] would seem to prove that the raising of the viscosity of the solution by the added extracts may be of equal importance.

Prof. Ide has evidently misunderstood the description of our technique when he gained the impression that the purity of our seedings was controlled by microscopic examination only after the first week. In our paper,[5] we state: "This successive seeding was continued to the fifteenth seeding with no apparent change in the rate of growth or diminution in the fermentative activity of the yeast. Every precaution was taken to insure pure seedings, a microscopic examination of a sample from each flask being made before seeding the next flask, because of the known influence of other fungi." By this we intended to convey the information that every one of the 15 consecutive seedings was tested microscopically in order to definitely establish the purity of the culture before seeding the next flask. The experiment extended over 3 months and a microscopic examination when the flasks were emptied showed no contamination.

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[4] Fulmer, E. I., Nelson, V. E., and Sherwood, F. F., *J. Am. Chem. Soc.*, 1921, xliii, 186.

[5] MacDonald, M. B., and McCollum, E. V., *J. Biol. Chem.*, 1921, xlv, 309.

In the studies reported by Souza and McCollum, and MacDonald and McCollum, it was not shown definitely what factor operated in the improvement in the growth of yeast through the addition of extracts of natural foods. In the recent publication by Fulmer, Nelson, and Sherwood, important observations are recorded bearing on this point. Their studies have shown that the concentration of ammonium salt which is optimum for the growth of yeast is identical with the concentration of the salt producing the least swelling of wheat gluten. They further observed that the addition of colloidal substances, such as starch or dextrin, so prepared as to leave no room for doubt that they were free from contaminating substances, exercised a markedly favorable influence on the growth of yeast. This affords convincing evidence that the optimum nutrient conditions for the development of yeast in media containing only known substances have not been appreciated or achieved by any foreign investigator up to the present time. The experience of Fulmer, Nelson, and Sherwood, correlates so perfectly and supplements so satisfactorily our own studies that we feel safe in asserting that it is fully established that neither the hypothetical "bios" nor the antineuritic or other uncharacterized dietary factor essential in the nutrition of mammals need be supplied in order to enable yeast to develop.

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THE ANTISCORBUTIC CONTENT OF CERTAIN BODY TISSUES OF THE RAT.

THE PERSISTENCE OF THE ANTISCORBUTIC SUBSTANCE IN THE LIVER OF THE RAT AFTER LONG INTERVALS ON A SCORBUTIC DIET.

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(Received for publication, September 23, 1920.)

One of the most striking features of the need of the animal organism for the group of unidentified food factors is the great dissimilarity which certain animals exhibit in their requirements as contrasted with the great similarity existing among others even more widely separated in the biological classification. For example the guinea pig and the monkey have closely similar requirements for the antiscorbutic substance. On the other hand the two rodents, the guinea pig and the rat, differ most remarkably in this regard. A discrepancy of this kind cannot but challenge investigation, because any light which may be shed upon the matter may eventually offer a clue as to the nature of the function of the unidentified dietary essentials in animal nutrition, and since one of the main objects of animal experimental feeding is to obtain a basis for judging the nutritive requirements of other species with which experiments are not so easily conducted, an understanding of any such pronounced differences in requirements as this is certainly to be desired. Several possibilities suggest themselves to explain the phenomenon. The antiscorbutic substance may be normally absent from the tissues of the rat, because the rat may not need this substance for the normal functioning of its body. The rat may require antiscorbutic substance, but be capable of synthesizing it. The antiscorbutic substance may pass into a modified form in foods on ageing, drying, etc. and this form may not be utilizable by the guinea pig but be utilized by the rat. The rat's requirement for antiscorbutic substance may be strikingly low. The rat's requirement for antiscorbutic substance may approximate that of the guinea pig from the standpoint of metabolism, but there may be less waste of the substance due to a different rate of excretion, etc.

The problem has been approached by Harden and Zilva (1) and by Drummond (2) from the standpoint of the susceptibility of the rat to an antiscorbutic deficiency as exhibited by differences in growth when an antiscorbutic food was added to, or withheld from, a purified ration. Distinct differences were noted in favor of the rats given the antiscorbutic food and Drummond concludes:

> "It may therefore be accepted as experimentally proven that the dietary requirements of the higher animals include in addition to a satisfactorily balanced ration of proteins, fats, carbohydrate and mineral salts, an adequate supply of three accessory factors: (1) fat-soluble A, (2) water-soluble B, or antineuritic factor, (3) water-soluble C or antiscorbutic factor."

The following ration is one fed to rats by McCollum and Davis (3).

Ration 223 B.

	per cent
Wheat	64.0
Casein	13.4
Dextrin	12.8
Salt mixture	4.8
Butter fat	5.0

It will be observed that this ration is closely similar in its constituents to the purified rations of Harden and Zilva and Drummond except that the antineuritic factor is furnished by 64 per cent of wheat instead of by the yeast. Small amounts of the other dietary essentials furnished by the remaining purified constituents of this ration, are also furnished by the wheat. Fully as excellent growth was obtained in rats by feeding this ration as by the use of the purified rations to which antiscorbutic food was added. Reproduction was also excellent. It is necessary, therefore, to assume either that 64 per cent of wheat in this ration supplied the rat's antiscorbutic requirement as completely as the orange juice or the treated lemon juice in the other diets; or that some other essential in the diet was supplied by or improved by the wheat addition. Until it can be proved that every individual factor in the purified rations used by Harden and Zilva and by Drummond are optimum in kind and amount except the antiscorbutic factor, there may still be some room for doubt whether the improvement resulting from the addition of fairly large amounts of complex mixtures such as orange juice and treated lemon juice is in reality due to its antiscorbutic content.

Plan of the Experiment.

The present investigation was planned to approach the problem from the standpoint of determining the antiscorbutic content of the rat's body tissues, (1) when a typical scurvy diet was fed to the rat, (2) when the diet was high in antiscorbutic substance. It was planned to test the antiscorbutic content by feeding the rat tissues or extracts of the tissues to guinea pigs on a scorbutic diet. Investigators (4, 5) had reported negative results in obtaining any beneficial effect when meat juice was fed to guinea pigs, so that presumably the rat muscle tissue might be indecisive for the test. The amount of this factor in body organs was apparently not so well established. A few preliminary experiments were therefore run with beef liver and pig liver, and the relatively high content of these tissues in the antiscorbutic factor gave promise that the rat liver would prove suitable for the experiment. The rat muscle tissue was included in the experiment, however, because in the negative experiments reported by others the freshness of the meat was apparently not insured, and it was thought that since ageing plays such an important part in the destruction of the antiscorbutic substance under certain conditions, this factor alone might account for the discrepancy of results between guinea pig experiments and the experience of explorers and soldiers (4, 6, 7). Also there was a possibility that if the presence of the antiscorbutic factor in muscle was demonstrated there might be a more pronounced difference between the two sets of rats in regard to the amount contained in the muscle than in the case of an organ usually containing a larger amount. Since it was easy to obtain fresh fish in the local market it was thought to be of interest to feed fish muscle at the same level of intake. In testing for the antiscorbutic factor by means of guinea pig feeding it is more nearly satisfactory to add the material to be tested to the scorbutic diet from the beginning of the experiment and to determine its effectiveness in preventing scurvy than to try to overcome it after the syndrome has developed. This, however, requires so much material, except in instances where the antiscorbutic substance is practically absent, that in the present investigation it was thought necessary to test the material by determining its effectiveness in curing the symptoms of scurvy in the guinea pig. An arbitrary standard of 15 days of feeding the material to be tested was adopted, as this had been found sufficient to insure the disappearance of signs of scurvy when sufficient amounts of pig liver were fed. In some instances treatment during this full time interval was not adhered to because of the limited amount of material.

Production of Experimental Rats.

Young rats about one-third grown were selected and fed for a period of from 213 to 247 days (depending on the time of using the individual rat for the experiment) on a soy bean ration similar to that employed by Cohen and Mendel (8) and later modified by Givens and Cohen (9).

Scorbutic Ration I.

	per cent
Cooked soy bean meal	76.0
NaCl	3.0
Ca lactate	3.0
Dried yeast	3.0
Butter fat	5.0
Dried skimmed milk	8.0
Filter paper	2.0

This ration was selected because it was desired to use one which was adequate for the nutritive requirements of the rat over long periods of time, and which at the same time was a satisfactory diet on which to produce scurvy in the guinea pig. Guinea pigs refuse to consume a satisfactory amount of many of the complex rations on which the rat thrives, so that the range of suitable scurvy-producing diets for use in guinea pig feeding is therefore narrowed. During 136 days of the feeding period 13 per cent of milk solids was incorporated in the ration. 8 per cent of dried skimmed milk was used, together with 5 per cent of butter fat to replace the fresh milk used by these investigators. Attempts to autoclave the dried milk resulted in such a degree of caramelization that this treatment was abandoned. During 38 days at the beginning, and from 39 to 73 days at the end of the experiment (the variation due to the time of killing the animals), the dried milk was left out of the ration altogether, and replaced by an equivalent amount of soy bean meal to eliminate the slight source of antiscorbutic substance in the dried milk.

Scorbutic Ration II (Ration I without Dried Milk).

	per cent
Cooked soy bean meal	84.0
NaCl	3.0
Ca lactate	3.0
Dried yeast	3.0
Butter fat	5 0
Filter paper	2.0

Production of Control Rats.

The control set of rats were full grown, vigorous rats which had been discarded from various experimental rations in the rat colony, and for a period of 18 days were fed a stock ration with 5 cc. of orange juice per rat per day together with a water extract of the peel of oranges, added to the drinking water.

Feeding of Guinea Pigs.

Except in the cases where it was known that the guinea pigs had been fed on an abundance of succulent food immediately previous to the experiment they were given orange juice for a preliminary period of 2 days. A longer period was not deemed necessary since it was intended to produce scurvy in the guinea pig as a preliminary to testing the rat tissues. Scorbutic Ration II, described above, was used as the basal experimental diet. It had been found with many experiments with these soy bean rations that when the soy bean meal is heated just enough to destroy the raw taste but not enough to brown the product markedly, the ration was eaten greedily by the guinea pig, but it was found that even though the autoclave pressure and length of time of heating given by Cohen and Mendel were strictly adhered to, the results could be made to vary from this standard of cooking acceptable to the guinea pig palate, merely by varying the amount of meal that was put into the autoclave at one time, or the surface exposed, as in flat containers. This may account for some unsatisfactory results that have been reported by investigators when using this ration.[1] With this proper degree of cooking always maintained the ration is eaten so greedily that the growth curves become a very helpful index of the degree of scurvy in the animal, for the stationary or declining weight of the animal is often the first warning of the onset of the syndrome and improvement in the symptoms of scurvy is nearly uniformly accompanied by an improvement in the curve of growth, the correlation of the two probably not depending upon any hypothetical growth-promoting properties of the antiscorbutic, but upon the fact that the guinea pig ceases to eat this ration when the symptoms of scurvy make eating painful and resumes eating promptly when the symptoms subside.

Preparation of Material.

The rats were killed by chloroforming, the day the tissues were used for feeding. The muscles used were those easily removed from legs and back. In order to make the most economical use of the material and feed it at a high average level, it was necessary for the amount of muscle used to vary somewhat from day to day, since the rats were not of uniform size. The muscle was reduced to a fine pulp with shears and extracted with distilled water for 3 hours by standing at room temperature. The residue from this was extracted again with distilled water for approximately 22 hours by standing at ice box temperature. The quantity of water used was two-thirds the weight of the portion of muscle except in the case of the two highest levels of feeding muscle, where it was found necessary to reduce this quantity on certain days with a variation in the capacity of the guinea pig to consume these large amounts. In the case of the butterfish, as much of the muscle as possible was removed from the bones, 35 gm. of this were weighed out, and an extract was made similar to that of the rat muscle. These water extracts were fed by means of a glass syringe.

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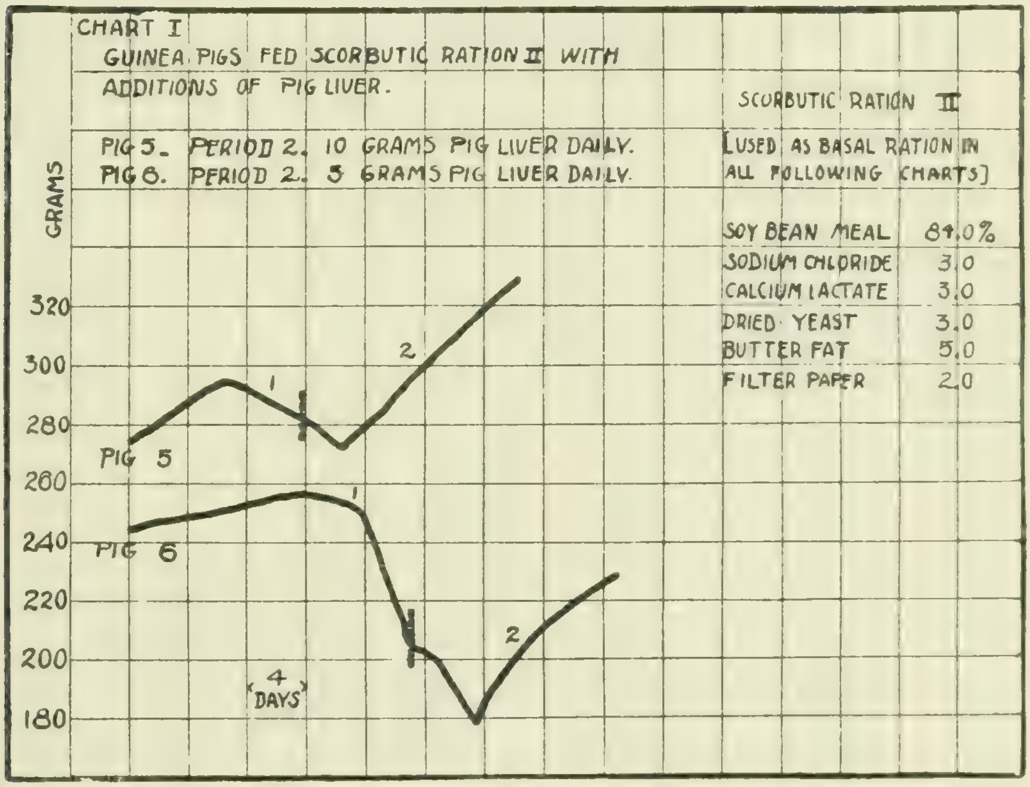
[1] Personal communication.

The given portions of the rat livers were weighed and rubbed through a wire sieve. This paste diluted somewhat with water could be readily fed from a glass syringe having a large opening. At times when the symptoms of scurvy in the guinea pigs were most severe it was necessary to strain out particles of the connective tissue with cheese-cloth as the least attempt at chewing appeared to produce much pain and a disinclination to take the material offered.

The results of the experiments are recorded in Table I and Charts I to VI.



SCORBUTIC RATION II
[USED AS BASAL RATION IN ALL FOLLOWING CHARTS]

SOY BEAN MEAL	84.0%
SODIUM CHLORIDE	3.0
CALCIUM LACTATE	3.0
DRIED YEAST	3.0
BUTTER FAT	5.0
FILTER PAPER	2.0

Chart I. The beneficial effects of feeding pig liver (Period 2) at sufficiently high levels to guinea pigs suffering from scurvy are shown. No. 5 on 10 gm. of liver per day made a complete recovery, but although improvement in the symptoms of scurvy of No. 6 was noted, apparently 5 gm. were not sufficient to insure the disappearance of the symptoms of scurvy in this time. The condition of scurvy in No. 6 was more severe than in No. 5, however, when the feeding of liver was begun. Although the pig liver was said to be fresh and not a cold storage product, the exact time that had elapsed since the killing of the animal could not be ascertained.

TABLE I.

Results of Experimental Diets.

No. of animal.	Day on which pronounced scurvy symptoms were noted.	Period described.	Tissues added to scorbutic diet of guinea pigs.				Day of experiment on which addition was begun.	No. of days addition was continued.	Remarks.	Autopsy.
			Animal from which taken.	Diet of this animal (or addition).	Tissue used.	Weight (or average weight) of tissues fed daily.				
						gm.				
21	10	2	Rat.	Scorbutic Rations I and II.	Water extract of muscle.	34	10	6	No improvement in scurvy symptoms on adding muscle extract to diet. Died on 18th day.	Severe scurvy.
		3	Pig.		Liver.	12	16	2		
26	19*	2	Rat.	Scorbutic Rations I and II.	Water extract of muscle.	38	13	12	Adding extract did not prevent incipient scurvy from developing into severe scurvy. Chloroformed.	" "
24	20	2	"	Orange.	" " " "	37	18	7	No improvement in symptoms on adding small amount of extract. Great improvement with larger amount in Periods 3 and 4. Chloroformed.	Some evidences of scurvy still present. Imperfectly cured.
		3	"	"	" " " "	55		8		
		4	"	"	" " " "	95		7		
30	18	2	Butter-fish.		" " " "	35	18	10	No improvement in scurvy symptoms. Died on 28th day.	Severe scurvy

5	10	2	Pig.		Liver.	10	12	14	Severe symptoms of scurvy disappeared on adding liver. Chloroformed.	Scurvy cured.
6	16	2	"		"	5	19	15	Very severe scurvy symptoms slightly improved on adding liver. Chloroformed.	Condition of scurvy still present.
20	10	2	Rat.	Scorbutic Rations I and II.	" kidney, spleen	18	10	15	Severe symptoms of scurvy disappeared on adding organs. Chloroformed.	Scurvy cured.
31	15	2 3	" "	" " " "	Liver. "	10 5	13	4 10	Moderate symptoms of scurvy disappeared on adding liver. Chloroformed.	" "
33	17	2	"	" "	"	5	17	11	Severe symptoms of scurvy greatly improved on adding liver. Chloroformed.	Lesions of scurvy almost disappeared.
27	16	2	"	Orange.	"	5	16	15	Severe symptoms of scurvy disappeared on adding liver. Chloroformed.	Scurvy cured.
34	18	2	"	"	"	3	18	15	Severe symptoms of scurvy disappeared on adding liver. Chloroformed.	" "

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* Scurvy symptoms not very pronounced.

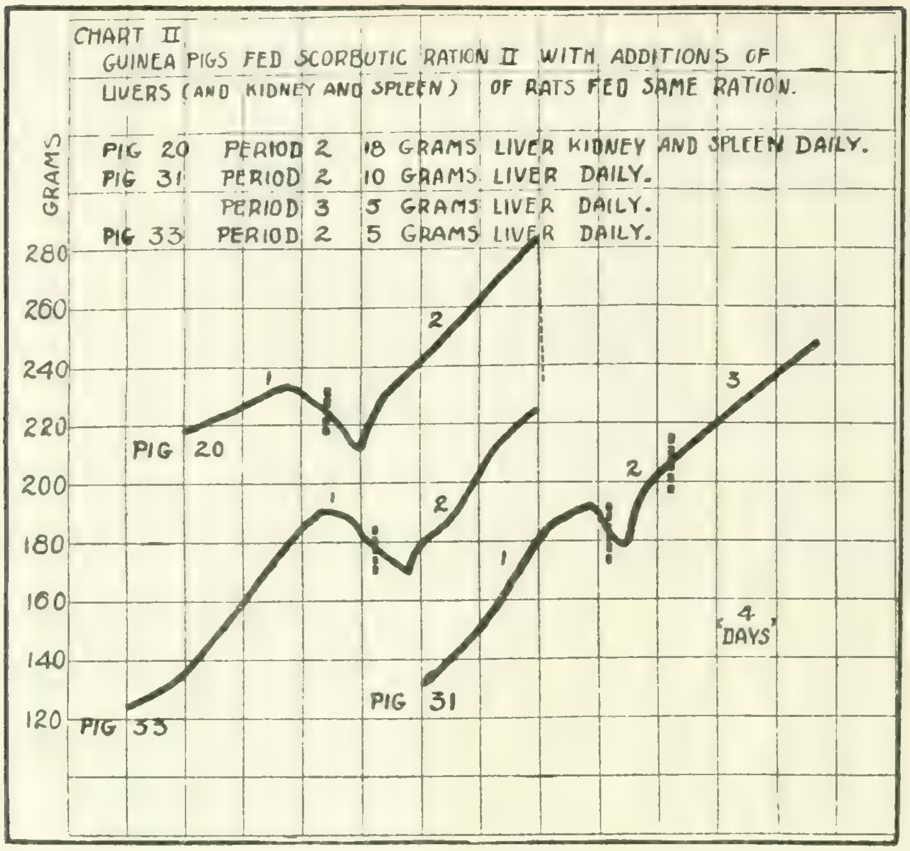

Chart II. This chart shows the beneficial effects (Periods 2 and 3) of feeding the livers of rats that had been fed a typical scurvy diet. In each of the three guinea pigs the symptoms of scurvy were severe and the recovery very rapid and marked. In the case of No. 20, the kidney and spleen of the rat were included with the liver to make the plane of intake higher, as it was very uncertain that there would be any considerable amount of the antiscorbutic substance in the tissues of the rats fed a diet in which it was so low. Towards the end of the experiment small rats were used and it was necessary to feed the organs of two rats instead of one on each alternate day to make the intake average 18 gm. daily. This high feeding on every other day produced on the last day of the experiment a digestive upset in the guinea pig with diarrhea, loss of appetite, and rapid loss in weight. No traces of scurvy appeared on autopsy, however, so that the symptoms had no relation to a condition of scurvy. Owing to the limited amount of material, No. 33 was fed liver for only 11 days. On autopsy slight traces of the scurvy condition were still present. These would undoubtedly have disappeared in 4 more days of feeding as completely as in No. 31 which received 10 gm. for 4 days and 5 gm. for the remaining 11 days. Apparently 5 gm. of this liver per day was not the minimum amount which would cure scurvy in the guinea pig.

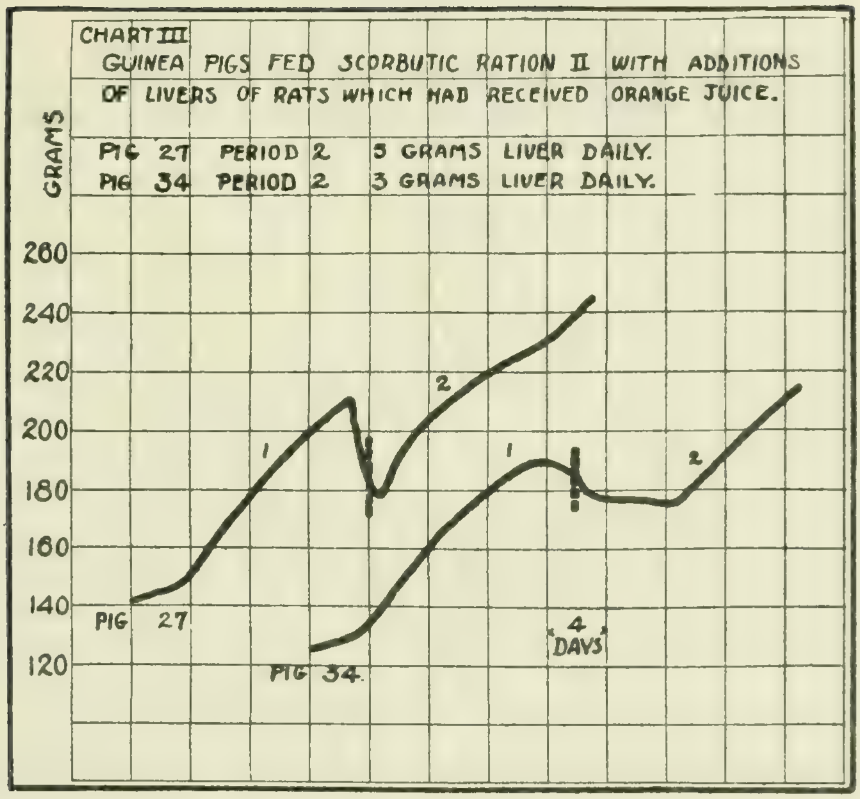

CHART III. The diet of these guinea pigs and those in Chart II are similar except that in Chart III the rats supplying the livers which were added during Period 2 were fed a large amount of antiscorbutic material in the form of orange juice for a period of 18 days previous to the experiment. A complete recovery was made in the case of both these guinea pigs but the recovery seemed to be somewhat slower in No. 34. Apparently 3 gm. of this liver a day is nearly the minimum amount which will produce a cure in this length of time. Compare these animals with those in Chart II where the rats supplying the livers had been fed the scorbutic soy bean rations.

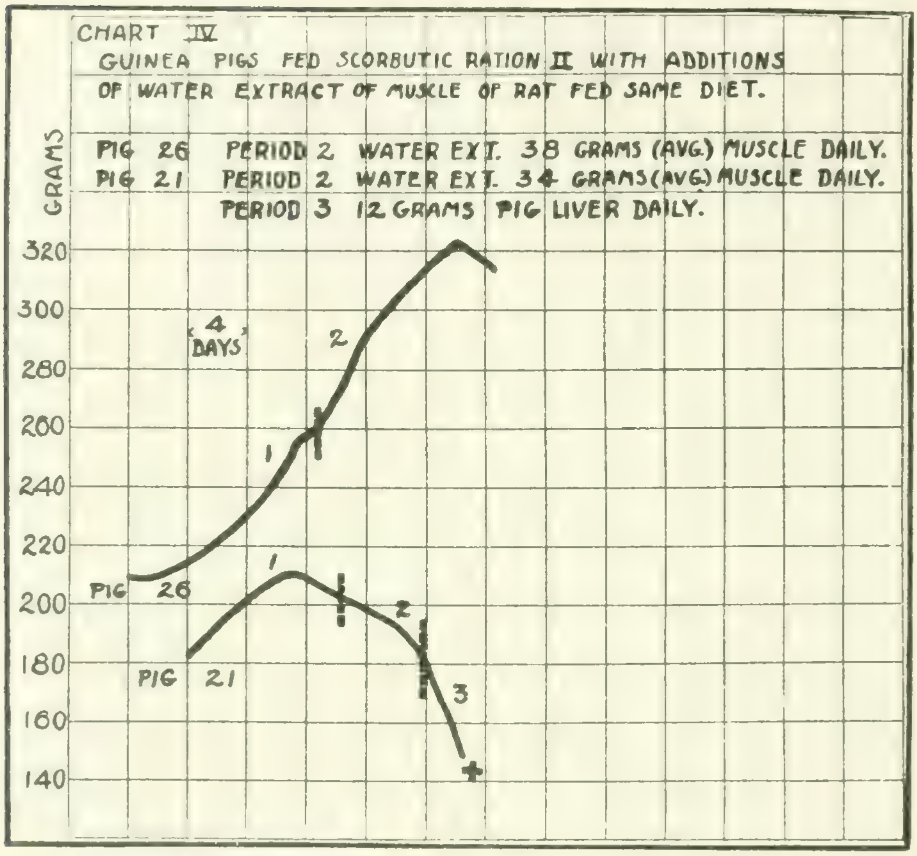

CHART IV. This chart illustrates the negative results in feeding guinea pigs water extract of the muscle of rats fed the soy bean diet. No. 21 was not benefited by 5 days of feeding this extract and was so weak and helpless at the end of this time that it took the extract with difficulty. An attempt was made to revive it by feeding pig liver but this failed and it died with severe scurvy.

No symptoms of scurvy had been observed when the feeding of No. 26 with this same water extract of muscle was begun, but these developed a few days later, and the guinea pig showed severe scurvy on autopsy. It was not possible to feed the muscle extract to this guinea pig at the same high level of intake as to No. 24 (Chart V), as it did not cooperate well in taking such large quantities.

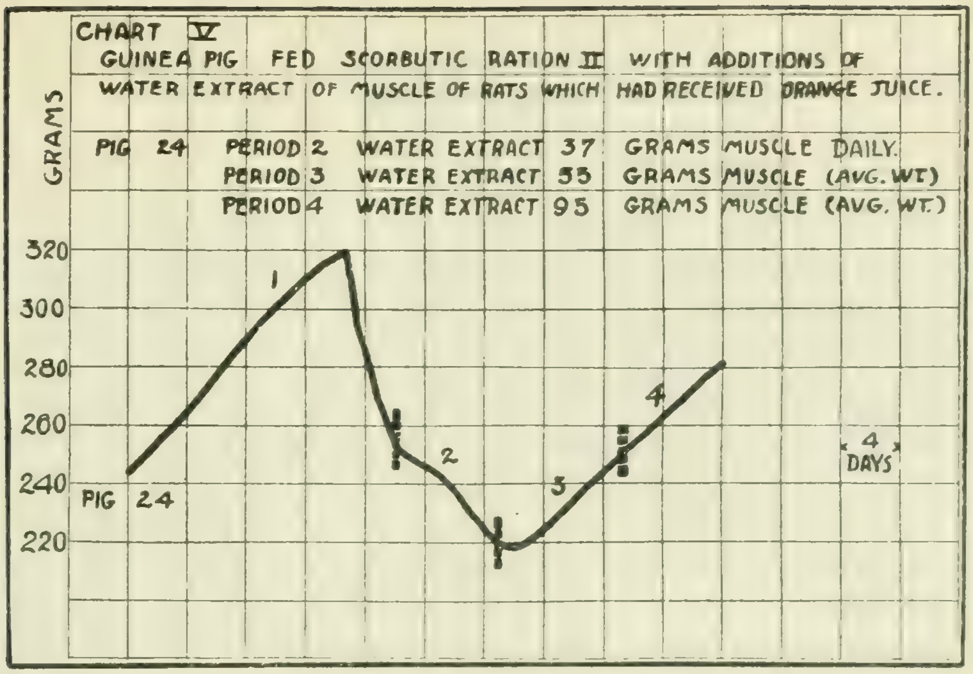

CHART V. This chart illustrates the effect of feeding a guinea pig water extract of muscle from a rat which had received orange juice. During the 7 days it was fed approximately the same level of intake that Nos. 21 and 26 received (Chart IV); the symptoms of scurvy grew steadily worse until the guinea pig was in a most miserable condition. However, it took the extract greedily so that it was possible to increase the amount fed. When the intake reached the equivalent of 55 to 95 gm. a day the symptoms improved, and the guinea pig was active and sleek when chloroformed. On autopsy, however, signs of scurvy were still present. Compare this chart with Chart IV.

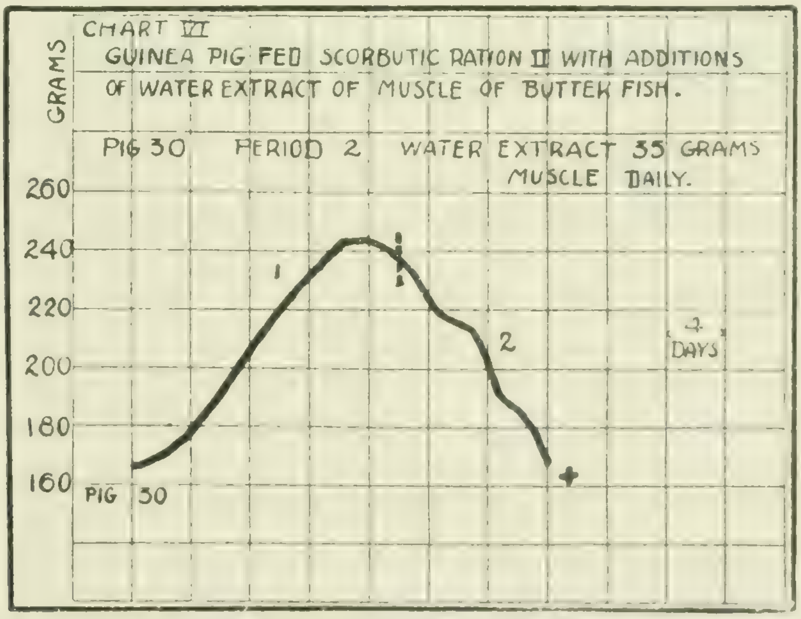

CHART VI. For purpose of comparison the water extract of fresh fish muscle was fed to this guinea pig at the same level at which extract of rat muscle was fed to No. 21. No improvement in the symptoms of scurvy was noted, but on the other hand the condition of scurvy grew steadily worse so that the pig died before the 15 days feeding was completed.

DISCUSSION.

The rapid disappearance of the symptoms of severe scurvy, substantiated by postmortem findings and correlated with the resumption of eating and a rapid gain in weight, during the 15 days (or less) when the guinea pigs were fed the livers of rats which had for a long period consumed a typical scurvy diet, leaves no doubt that this tissue with this history is rich in the antiscorbutic factor. It was, in fact, effective in relieving the symptoms of scurvy at such a low level of intake (5 gm. during a period of 11 days) that, without the use of larger numbers of animals, it could not be said that the livers of the control set of rats which had consumed orange juice were richer in this factor. This similarity between the two sets of rats in their tissue content of antiscorbutic factor is not contradicted by the results in feeding the muscle although the results in this case are indecisive, since it was not possible during this investigation to feed both at the same excessively high level. These results are in agreement with the instance cited by Smith (6) of the Arctic explorer, Dr. Kane, who attributed his freedom from scurvy during a winter on shipboard in the Arctic regions to the fact that he ate the rats with which the ship was infested. The diet upon which these rats lived must have been very low in the antiscorbutic substance.

This demonstration of such considerable amounts of the antiscorbutic factor in the livers of these rats disposes fairly definitely of the first hypothesis stated; namely, that the rat may not require antiscorbutic substance for its normal metabolism. It seems entirely improbable that the presence of this factor in the bodies of these animals should be accidental, especially since the amounts in the bodies of the two sets of rats approximate each other so closely. A synthesis of this factor is indicated, both by these results, and by the excellent growth and reproduction obtained in rats by the feeding of such rations as Ration 223 B cited above, in which no constituent has been shown to allay scurvy symptoms in the guinea pig. Closely allied to this hypothesis of a synthesis is the possibility that the rat can utilize some slightly different inactive forms of the antiscorbutic substance into which the active form might theoretically pass on ageing, drying, etc., and from which it might be rapidly changed in such physiological processes as the sprouting of seeds.

An alternative explanation is that the rat has a very definite food requirement for the antiscorbutic factor but that this is quantitatively very small in comparison with the requirement of the guinea pig and has been met in this experiment by the content of the soy bean rations used. This hypothesis might explain also the success of such rations as Ration 223 B. Some constituent of this diet might presumably carry a sufficient quantity of the antiscorbutic factor for the requirement of the rat, but escape detection in the light of the much larger requirement of the guinea pig. But if this explanation is accepted it is surprising to find such an abundance of the factor in the bodies of the rats fed on the scorbutic soy bean diet. It would be logical to assume that an extremely small requirement for a food factor would not necessitate the presence of a relatively large supply of that factor in the body of the animal. It is conceivable that the rat has acquired not only a lowered requirement but also a phenomenal capacity to store the antiscorbutic substance through a biological adaptation to a food supply which over long periods of time is very deficient in this substance. It is not apparent what relation the presence of this factor in the liver bears to its abundance or depletion in the rest of the body. It is conceivable that the liver cells are so constituted that a considerable supply of the antiscorbutic factor may be contained in them while at the same time the content of other tissues is below the level at which proper functioning is possible. It is proposed to test this point by determining also the antiscorbutic content of livers of guinea pigs suffering from scurvy as contrasted with the livers of normal guinea pigs. Until such a determination is made, the most probable hypothesis is to assume that such a strikingly large amount of the antiscorbutic factor as has been demonstrated in the livers of these rats indicates a satisfactory content in the body. It remains to be seen what amount of antiscorbutic substance is contained in the tissues of rats fed over long periods of time on adequate purified rations, freed from the antiscorbutic factor as thoroughly as can be done in the light of our present knowledge. This freedom is difficult to insure, as the guinea pig's requirement is so high that it is impossible to detect the presence of traces of antiscorbutic substance in their diet. In an otherwise purified diet the material carrying the water-soluble B might be under suspicion of also carrying traces of the antiscorbutic agent. The work of Delf (10) makes it uncertain also just what degree of heating would be necessary to insure that no trace of this substance would be left in a treated food.

Preliminary experiments of an investigation aimed to test the comparative amount of antiscorbutic substance in eggs and in newly hatched chickens appear to give some additional weight to the probability of a synthesis of antiscorbutic substance by certain animal tissues, or a utilization of forms other than those available to the guinea pig.

SUMMARY.

1. A high content of the antiscorbutic substance is demonstrated in the liver tissue of the rat not only after feeding for a short time on a diet rich in the antiscorbutic substance, but also after feeding over a long period of time on a typical scorbutic diet.

2. These results are offered as an indication of the need for the antiscorbutic factor in the normal metabolism of the rat.

3. Possible sources in the rat's body of the antiscorbutic substance, such as the presence of undemonstrated amounts in the food, utilization of a form not available to the guinea pig, and a synthesis of this factor by the rat's tissues, are discussed.

BIBLIOGRAPHY.

1. Harden, A., and Zilva, S. S., *Biochem. J.*, 1918, xii, 408.
2. Drummond, J. C., *Biochem. J.*, 1919, xiii, 77.
3. McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, xxi, 615.
4. Chick, H., and Hume, E. M., *J. Roy. Army Med. Corps*, 1917, xxix, 121.
5. Dutcher, R. A., Pierson, E. M., and Biester, A., *Science*, 1919, l, 184.
6. Smith, A. H., *J. Roy. Army Med. Corps*, 1919, xxxii, 188.
7. Stefánsson, V., *J. Am. Med. Assn.*, 1918, lxxi, 1715.
8. Cohen, B., and Mendel, L. B., *J. Biol. Chem.*, 1918, xxxv, 425.
9. Givens, M. H., and Cohen, B., *J. Biol. Chem.*, 1918, xxxvi, 127.
10. Delf, E. M., *Biochem. J.*, 1920, xiv, 211.

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FOOD AT THE FRONT LINES

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EARLY in 1918, five officers who were experts in food problems, were sent to France. Under the command of Maj. P. A. Shaffer, San. C. they were assigned as the Food and Nutrition Section, Medical Corps, to the Division of Laboratories and Infectious Diseases, which was in reality one of the major subdivisions of the Division of Sanitation, Office Chief Surgeon, A. E. F. On the basis of their recommendation twenty-five more officers of the Food and Nutrition Section and sergeants of the Q. M. Corps were sent to the A. E. F. The phase of their work dealing with combat troops is told here, based on experience in the 1st, 2d, 3d, 4th, 5th, 6th, 7th, 26th, 28th, 29th, 32d, 33d, 82d, 87th, 88th, 89th, 90th, 91st Divisions and the Corps Headquarters of the 1st, 3d, 4th, 5th, 6th, 7th, 8th, and 9th Corps.

Parties were attached on more or less permanent duty with corps or divisions. The parties consisted of an officer of the Food and Nutrition Section and several non-commissioned officers. They were in close coordination not only with the Headquarters, Division of Laboratories and Infectious Diseases by telephone and telegraph but also with division officers, especially Division Surgeons, Sanitary Inspectors, G-1 representatives of the General Staff, General Inspectors Quartermasters, and organization commanders. This allowed them to use their own resourcefulness and give such advice, make such plans and assist in carrying out such actions as the immediate occasion demanded. The work called for considerable detailed inspection of kitchens and instruction of the personnel individually, in groups, and in schools. As was to be expected the problem in each division was different but the main work was to see that the men received the food under the best possible conditions.

The nature of the ration in kind as authorized by G. O. 176, A. E. F. series 1918, cannot be discussed here. In general the garrison ration was consumed unless the field or other ration was designated by the commander. There were two days' reserve rations, which each soldier carried on his person. In addition, the unit combat wagon carried two days' field and one day's reserve, and the Divisional Supply Train two days' field rations. This made three reserve and four field, enough, if intact, to subsist the command for one week. These rations were never used unless a real necessity arose, for new rations were continually sent forward. While occupying trenches the soldiers were entitled to extra trench allowances in coffee, sugar, meat, bread, and candles. It can be said that the food supply for troops in the A. E. F. was of uniform excellence and, with certain gaps and difficulties, always adequate.

THE GARRISON RATION

Meat.—The meat component was very well handled. Young, fresh, frozen, prime steers were delivered to the kitchens in better condition than to the average American home. With combat troops no inferior or spoiled meat was seen; a small amount needed considerable trimming. The supply was excellent and plentiful. Bacon was appreciated up to 20-25 per cent of the ration; greater amounts could not be utilized and found their way into the fire. At times no other than canned meats were available—roast beef, corned beef and corned beef hash. The canned meats were satisfactory and the amount issued was not excessive. Salmon was an article of food in which considerable wastage occurred; the American soldier will not eat more than a limited quantity of "Goldfish" (salmon).

Bread.—At all times the white bread and white flour were a source of wonder to the poilu as well as to mam'selle and Fraulein. The bread was the regular garrison (white) 2 lb. loaf. During the summer of 1918 a considerable amount of bread was lost, due to an epidemic of mould which resulted apparently from methods of handling, packing, and shipping in the French "40 hommes 8 chevaux" cars. Many organizations suffered, as the loss amounted to 100 per cent in some cases, and there was nothing to replace it. After the four pound oval loaf was substituted very little difficulty was experienced. We have seen this bread issued three to four weeks after being baked and still in good condition. Later the small, round, two pound loaf from the mechanical bakeries at Is-sur-Tille was also received and gave satisfaction. As for the hard bread, that packed in tin containers was very satisfactory; that packed in cardboard or the French variety packed loose in crates suffered considerable loss through moisture and mould.

Vegetables.—Fresh vegetables were often insufficient They were substituted in part by dehydrated vegetables or by fresh vegetables bought locally. All the officers reported that both these alternatives caused trouble. The cooks had never seen dehydrated vegetables and had never, of course, received any instructions in their preparation. In some places no water for soaking them was available. As a result they were badly cooked. The fresh vegetables bought from the French and sent by the Regulating Officer, while very acceptable, were an almost total loss. The fresh tomatoes and cabbage especially, when they reached the front lines, were only 5-40 per cent fit for food. The fresh vegetables bought locally were of course much better. Fresh vegetables were also available in rapid advances. In the Argonne large cabbage fields were a great help in supplementing the diet.

Fruit, milk and cereals were very short, both as allowed by the ration and as actually received. The pepper accumulated. The lard was excessive and went into the fires as an almost universal practice.

Other Rations.—When troops were in combat or advancing to combat the field ration was designated by the commanding officer. This consisted of canned meats in place of fresh and hard bread instead of soft. From the soldiers' point of view, it was otherwise indistinguishable from the garrison ration. The reserve ration carried on the soldier and in the field train, consisting principally of canned meat and hard bread, was never to be eaten except in an emergency; but practically, it was quite surprising how readily the American soldier made the emergency and the avidity with which he consumed a full day's reserve for one meal. The special trench reserve ration consisting of 25 rations sealed in metal containers was designated for trench warfare. It was meant to be consumed following a heavy barrage when rations could not be brought up to the men. Such conditions did not arise in the American Army and this ration was never utilized. The travel ration consisted of canned meat, soft or hard bread, canned beans, and canned tomatoes; sugar and coffee being usually supplied en route. This ration on the whole was satisfactory except for the supervision of its use in the trains. It was the custom for each man to open a can and eat until the contents were consumed. A sergeant should have been placed in charge of all the food in each car and have served it as needed.

The garrison ration delivered to the A. E. F., both as to quality and quantity marked an epoch in the feeding of armies. In many respects the ration was ideal. Probably better results are attainable in the milk and vegetable components by dehydration. The meat and bread components were present in such vast amounts and of such excellent quality that in this respect, the American food supply probably surpassed that of any previous army. The ration as delivered to the combat troops in France, in spite of decreased tonnage, submarine warfare, limited railroad transportation, storage, motor truck shortage and other difficulties was the envy and admiration of the Allies and a triumph of American achievement.

SUPPLY WITHIN THE DIVISIONS

The functioning of the supply and the system of automatic issue cannot be gone into here. Briefly, food moved through France from Base Depots to Intermediate Depots; from Intermediate Depots to Advance Depots; through the guidance of the Regulating Stations to the Railheads and from the Railheads to the Divisions. The divisional supply organizations delivered the food to the separate organizations. The problem of supply within an organization caused more trouble than was anticipated. During the period at the front when accountability was removed, the supply officers, organization commanders, and mess sergeants assumed that the ration was: "What-ever we get." In many cases losses were not made good, spoiled goods were not redeemed and rations were sent to feed twice as many men as they were intended for. Part of this difficulty arose from the shortage of transportation. Part arose from petty thefts along the line of supply. No ration slips were available to check the supplies so that the more desirable articles had a tendency to disappear before they reached the front lines. The guards, stragglers, K. P.'s, etc., ate what jam and butter escaped the Officers' messes.

Ration Reports.—Ration reports were also responsible for certain delays in transportation and in getting the food to the men. It was customary for the daily strength report to be sent to the Battalion Headquarters, there consolidated and given to the Regimental Headquarters where it was again consolidated and given to the Supply Officer. The Supply Officer took the report to the Railhead as a basis for the ration return. The food was not issued until the next day and was not delivered until the third day when it was in the hands of the Regimental Supply Officer to be distributed to the Companies. The officers of one Division suggested that this line of communication could be reduced considerably by telephoning the daily report to the Railhead for issue the same day. This brought about considerable improvement because it eliminated the shortage of food resulting from detachments continually arriving at the front. These were often without food for three days unless they could take part of that meant for the troops already there. In the rarest instances did the Commanding Officer make provision for detachments sent forward.

The system for food supply was the daily issue. This allowed quick moves without involving much transportation of food. As can be seen from the following quotation of Maj. P. A. Shaffer, it was not without its difficulties.

"With the daily system of issuing rations now being adopted, I should advise that the Division Quartermaster should be allowed three days' rations to meet the delay in receipt of rations, and in order to minimize the breaking of packages. The division of sacks of flour, sugar etc., is wasteful and unsanitary, and should be avoided by the 'under and over' system of daily supply from the Division Quartermaster or from the regimental supply points.'

"Also, in my judgment, with the daily issues the whole of each ration article or the whole allowance of a substitute should be issued to an individual mess, rather than a part of ration article and part substitute. For instance 60 per cent beans and 40 per cent rice gives not enough of either for a meal and makes the making of menus difficult."

STOVES

No description of the food conditions would be adequate without consideration of the stoves for cooking. Many of the troops, when at the embarkation camp, had their field ranges taken away from them and on landing in France were practically without cooking equipment of any kind. This was all the more serious in the case of the National Army Divisions since what training the cooks had had in the cantonments was on hotel ranges. The Officers from the Food and Nutrition Section assigned to Divisions tried to assist them to make temporary substitutes such as rock ovens and mud ovens until rolling kitchens were available. There were four main types of rolling kitchen with which the doughboys struggled in the A. E. F.: the Magor, Steinbach, Taylor No. 29 and Taylor No. 30 and none was satisfactory. A detailed analysis of their shortcomings can not be given here. In the Army of Occupation a new type of rolling kitchen was provided and was far superior to those mentioned above.

The objections to the rolling kitchen are many. A good fire cannot be maintained and they can not be used for cooking while in motion. They turn out poor meals, as owing to the absence of ovens or poor ovens, the cooking is practically limited to frying and boiling. In service they have shown to be very hard on men and horses. They transport poorly especially when trailed behind trucks—the fire box breaks and the stoves shake apart.

The Army field range with the Alamo attachment has proved its advantage over all types of rolling kitchens. The men could set it up and cook a meal almost as quickly as they could prepare a meal on a rolling kitchen. It has good ovens. It can also be installed as a permanent kitchen and as such gives excellent satisfaction. During the time when troops are in trench warfare, in support, in reserve, or in rest, this type of kitchen is superior in every way. During a rapid advance there is a time when the rolling kitchen is invaluable; however, even under these conditions it is far from ideal. In the Argonne the kitchens were easily spotted by the enemy due to their smoke. As a consequence they drew heavy shell fire and many direct hits. The result was that the kitchens were grouped far behind the lines and the food had to be carried a long way. There is an important *but limited* use for any present type of rolling kitchen in combat.

MESS CONDITIONS

The great part of the work of Food and Nutrition parties was to improve mess conditions in the field. This required a major portion of the time of officers and sergeants. The policy was not to make an inspection followed by a report with only destructive criticism. Every effort was made to find out what was the matter with the particular mess and to give such advice and information as to overcome the difficulties. A great deal of time was spent in the individual companies instructing them in the elements of mess management, drawing of rations, storage, and in elementary problems of cooking and sanitation. In this way the officers were able to show the company commanders where the fault lay and the sergeants were able to give such instructions as would improve the cooking and reduce the waste. Small groups of mess sergeants were given informal lectures; circulars of instruction were issued for general distribution, temporary schools were put into operation. In addition the officers had many conferences and made such reports as were necessary.

Handling the Ration.—The training of the mess sergeants to meet actual conditions in the A. E. F. was very faulty. Nearly all were trained in the Cooks' and Bakers' Schools. A considerable portion of their time in cantonments and posts at home was spent in the purchase of food in the local markets and in the bookkeeping involved in the ration savings account. They did not know the ration allowance. They did not know how to make good their shortages. Few knew anything about the proper setting of a field range; none knew anything about the rolling kitchen. They were not trained in the proper handling of food under field conditions. They had not received sufficient training in field sanitation. They did not plan or keep their menus but turned over the daily supply to the cooks and K. P.'s. Their system of training resulted in the mess sergeants doing very little and using their time to poor advantage.

Cooking.—As far as making of menus is concerned, little was possible in a good many cases. It was sometimes merely a process of turning the food into "slum." The cooking in the A. E. F. was on the whole only fair. Not enough cooks were available to meet the demand of the Army. Cooks require considerable training, even with the best instruction, for proficiency. Too often these men were selected to be cooks because they had flat feet and were not available for line duty. They had never received training in field cooking or with the ration in kind. They had never seen a rolling kitchen until they were issued one as they went into combat. Then they were expected to cook without any ovens or griddles. The stoves broke and burned out, the utensils were often short especially after a few months of field service and several troop movements, and replacements could not be obtained. The cooks had never seen dehydrated vegetables until they were issued in the A. E. F. and received no instructions in their preparation. These were badly prepared and the men were thus prejudiced against an excellent article of food. Fuel difficulties of green wood or no wood added to the cooks' troubles.

In the A. E. F. there were four cooks to a company; these were on duty twenty-four hours and then off duty twenty-four hours—two to a shift. It is felt that better results would be accomplished if the two cooks who get up for breakfast come on duty the next day in time to assist in serving the noon meal. Cooks should be trained to work under the conditions they must meet in combat.

Serving of Food.—Not enough time and attention were given to the serving of food. In rear areas a man should not be compelled to stand in line forty-five minutes to an hour before he gets his food. It cuts down his time for rest and recreation. If a company can be fed in a double line in three to five minutes, as it should be, it solves not only that problem but also the question of "seconds" getting in before the line is served. If all the cooks are on duty at meal time there would be better slicing and serving of meats and better seasoning of food.

In the front line the practice was to send the food up at night in Marmites or insulated cans. The British, French and German food containers were all designed to feed a squad or small body of men. The designers of the American food containers must have thought the men came to their meals in "company front" formations. The containers should have been much smaller so as to have been more easily carried.

Sanitation.—In general the kitchen sanitation was satisfactory in so far as the health of the troops was concerned but left much to be desired from the point of view of cleanliness and esthetics. The beef was piled up on the ground or on boards instead of being hung uncovered and trimmed. The bread was often put on the ground instead of being in bread boxes or the sacks hung up. The same is true of the bread crumbs. The sugar and flour were not in rat or vermin proof containers and to our surprise no loss from this source was reported. They did, however, often get wet. The vegetables were not so arranged that they could be picked over. In general mess kits were well washed. The garbage was not excessive in the A. E. F. and in several organizations well trained in this respect, as the 88th and 91st, waste was very small indeed.

Supervision of Mess.—An Army kitchen requires constant supervision to attain best results. This was supplied in the States in part by the Cooks' and Bakers' Schools. The transient and infrequent inspection by a nutrition officer had limited value. Constant attention to details is essential. Line officers and medical officers made such inspection as their time and qualifications permitted. The line officers who recognized the importance of the mess were usually entirely dependent upon the mess sergeant. Instruction and experience for line and medical officers in the principles and practice of cooking and serving of food would give them the technical knowledge necessary for intelligent supervision. This would permit the nutrition officer to aid them with their problems rather than to bring to their attention the elementary points. Many officers did not give the proper relative importance to the kitchen. They choose their mess sergeants because they were unsuited for the line to the obvious harm of the mess. They also were so busy with the many details of training, equipment, maneuvers, etc., that they spent their time on these problems, forgetting that the men fought once in a while but ate three times a day.

TROOPS UNDER MOVEMENT AND COMBAT

Advancing from a rest area to the front is usually accomplished with little trouble—the ground has all been marched over before, villages marked for billets, railheads already located, and rapid handling of rations made possible. The method used in such an advance is quite simple. Usually Divisional Headquarters moves once in two days. The same is true of the Railhead, which one day is in advance of the moving division, the next day in its rear. It means the supply train hauls one day to the organizations in the rear of the dump and the next day to the same organizations in advance of the dump. When secrecy of movement is a factor troop movement is accomplished at night. This "leap-frog" system allows of a semi-stationary headquarters and ration dump while the division continues to advance. Arriving at the front the advance railhead is passed and a ration dump established at some point further forward while troops pass to the line. In such a situation rations are hauled by truck from railhead to dump. In a country with many intersecting railroads as in France this is not usually a long haul; but when the roads are destroyed, as occurred in the Belgian Campaign, it may be necessary to haul by truck for 60 kilometers.

The actual food conditions in combat can probably be told best by the following quotations from reports of officers made on the spot.

ST. MIHIEL

"There is needed, especially in the line companies, a closer co operation between the Supply Officer of the Regiment and the various kitchens of the organizations. In some cases the food was delivered all right to the kitchens, but the proper guide was not furnished the kitchen authorities, so that in some cases the food did not reach the men in action. At one Regimental food dump visited by our field party, food was piled in equal numbers of supplies regardless of the number of men in each company to be fed. Also the division of rations was not plainly separated, so that one organization did not know which was their share or where their share left off. No regimental issue slips were signed or given out at this particular regiment. Nevertheless the men in the 26th Division did not go hungry after obtaining their objective in this particular drive on the German trenches, because vast quantities of German foodstuffs were captured, such as oatmeal, unground coffee with roasted barley, soup in cubes two inches square wrapped in waxed paper, wrapping paper outside. The soup cubes consisted of cooked, dried vegetables pressed together. In case of beans, the beans evidently were previously cooked, dried, then powdered and pressed into cakes. Fifteen minutes cooking, according to the label, prepared it for use. The German fresh bread was dark in color and of a sour taste and odor. The bread appeared to be made of rye and graham flour, loaves being about fourteen inches long and six inches in diameter, weighing between two and three pounds per loaf. The hard bread was in small cubes about three-quarters of an inch long and one-half inch wide, slightly sweetened and of delicious taste. The Americans also captured and consumed a considerable amount of German canned beef, many barrels of beer, condiments such as dill pickles and other German food."

"Found by inquiry that two days' reserve had been issued for each man of reserve rations and that one day's reserve and two days' field was in the field train and the two days' field was held by the Quartermaster. From the talk of the infantry at the front the day before the drive started I judged that a great many men discarded their two days' reserve so as to be able to travel faster and not be hampered by any 'unnecessary' equipment. The day after the advance started the Division Q. M. moved the ration dump up to the town on the old line; from there the Supply Officer sent out trucks to the advanced troops. So rapid was the advance that the Supply Officer could only designate to the driver the distribution of the rations in the most general terms; they had completely lost liaison with their organizations and it seemed highly improbable that the ration trucks would find and deliver the rations to the unit for which they were intended. The congestion of all the main roads was very bad, the rations were delayed eight to ten hours between the railhead and the Divisional Dump with other traffic. On every side I saw rolling kitchens doing splendid work feeding whatever troops were in the vicinity. Field ranges were also hastily set up and in use but it seemed to me that the rolling kitchen was by far the most practicable and serviceable method of feeding troops. With the congestion of traffic on the roads that seemed inevitable during a rapid advance it would seem necessary for organizations to depend for a few days on their reserve and field rations carried by the men and the field trains. Conditions of traffic and poor liaison are such as to indicate that supplies sent from the railhead or Divisional Dumps would not reach the troops for which they are designated for several days."

ARGONNE

"In spite of frequent movements, the railhead office and supply train have been functioning admirably though under great difficulties; there is not a full quota of trucks and since the recent advances the ration dump had to be changed frequently. In spite of this the food has always been on hand, distributed to the supply companies of the regiments and by them to the kitchens. The rations were unloaded at the railhead, through it to the ration dump and from there distributed to the kitchens. The kitchens were all together. They should be kept together, at least for a Battalion, the stoves kept more or less permanent, letting the front relief carry the cooked food three or four miles if necessary, but not further. In this way the kitchens would not become lost as happened more than once."

"On the first troop movement the soldiers of one regiment did not carry rations with them as they were instructed and so were without food. Again this was a common difficulty; for three days of the advance most of the men were not receiving any hot food, the kitchens had it and cooked it but were unable to find the men."

"Most of the organizations in the front now can only receive food in the night, they eat two meals a day and the food has been ample for these two meals. There has been an occasional shortage of some article of the ration due to the fact it had not been received at the railhead but on the whole the men have fared very well. When in reserve the companies have access to the rolling sales commissary where they buy oatmeal, jam, etc. One trouble in the front is the shortage of water and particularly for this reason it is hard to utilize dehydrated vegetables, fortunately fresh vegetables have been issued frequently during the past week."

"Due to the difficulties of the attack and progress afterwards (shell fire, bombing, rain, etc.) it was practically impossible to get any food forward for 36 hours—that is late on the afternoon of the 27th of September (the attack commenced at 5:30 A. M. on the 26th) when wagons were pushed ahead with corned beef, baked beans, hardbread and jam.

Due to the crowded conditions of the roads, filled with ammunition trucks, advancing artillery and ambulances no rolling kitchens could be carried forward for some time. It became necessary for the men to subsist on what they could get, and as most of the soldiers, as before stated, had eaten the entire reserve, they were hungry before food could arrive. As the attack gradually developed into an enemy retreat, order began to come out of the first few chaotic days, the roads were rapidly repaired and within four days rations were going forward quite regularly —canned meats, potatoes, beans, coffee, milk, etc. No fresh beef and no bacon were received until the division was withdrawn, when the beef component arrived, but bacon was not received by the division until late in November, nearly two months."

"Kitchens were placed in any sheltered location available. The military phase of these movements were preparations for a surprise attack. Men bivouacked in the forest of Hesse—under cover, all smoke was avoided—all lights at night were carefully covered. Usually cooking was done in late afternoon and the food sent forward in marmites. Some kitchens were two kilometers from the organizations, others closer, while one kitchen was located in a cave within five hundred meters of the trench. Rolling kitchens had been supplied by this time, but most cooks preferred to set up and use the field range. The food offered little variety; dehydrated vegetables, corned beef, bread, jam, dried fruit and coffee; these were the chief items."

"During this time considerable attention was given to the reserve rations on each man, it being desired that no man go over the top without the reserve. One of the striking things about the American soldier is his utter disregard for the future as shown by the avidity with which he will eat two days' reserve ration for lunch on the first day, throw away his slicker the minute the sun appears, chuck his blankets when going gets hard, and then go hungry, cold, and wet and cheerfully blame the whole affair on someone else."

From the nutritional point of view it is an interesting fact that the men under actual combat conditions as in the Argonne Drive, when it was not possible for kitchens to be brought up nor food to be supplied and when they received only one meal a day, did not complain seriously about being under nourished. Part of this may be ascribed to the excitement of battle and part to the splendid morale of the American soldier. However, these conditions of rapid advance and intensive open fighting were really only in evidence in the Argonne. The actual time spent under these conditions was short and unimportant from the physiological standpont. No one suffered from lack of food. Not forgetting the minor mistakes and difficulties there is no other possible conclusion to draw than that the American soldier received a splendid ration, of excellent quality and always at hand.

SUMMARY

1. *The Reserve Ration* is at present unsatisfactory. It wastes food as hard bread and does not contain the proper components for a ration to be carried by the soldier. A permanent package that will fit in the haversack containing several articles, constituting one complete meal,—three to the ration—is urgently needed.

2. *The Rolling Kitchen* should be greatly modified or superseded. It must, 1st be as usable as the field range, 2d, be motorized, 3d, be rubber-tired, light, and set on springs, 4th, be equipped with sufficient utensils, including a griddle, oven, meat and serving table and containers capable of withstanding heat, and 5th, be oil burning to avoid being detected.

3. *The Marmite Cans* or food containers should be of size sufficient to feed a squad or small detachment.

4. *Mess Sergeants and Cooks* were not selected with due regard to their ability or qualifications. Their training was not such as to fit them for greatest usefulness under combat conditions. They should receive better instructions in the use of the ration in kind and the messing of troops under field conditions. K. P.'s should not be changed daily and kitchen police duty should not be used as a punishment.

5. *Commanding Officers* paid too little attention to the kitchens, were not thoroughly conversant with the ration, and were inclined to let the Mess Sergeant struggle on. As now organized a Company Commander and his officers had a great deal to do, the company was large, and men had to be trained for fighting. The kitchen suffered. Too often inspections were cursory, resulting only in destructive criticism. Company commanders should receive instruction in the feeding of men and mess management.

APPENDIX

G. H. Q., AMERICAN EXPEDITIONARY FORCES, GENERAL ORDERS NO. 176.

FRANCE, *Oct.* 11, 1918.

1. On and after November 1, 1918, the kinds and quantities of the components of the rations and the substitutive equivalent articles which may be issued in place of such components to troops of the A. E. F. shall be as follows:

2. THE GARRISON RATION

COMPONENT ARTICLES AND QUANTITIES		SUBSTITUTE ARTICLES AND QUANTITIES	
Beef, fresh	20 oz.	Mutton, fresh	20 oz
		Beef, fresh, boneless	16 oz.
		Bacon	12 oz.
		Pork, fresh	16 oz.

COMPONENT ARTICLES AND QUANTITIES		SUBSTITUTE ARTICLES AND QUANTITIES	
		Sausages, canned pork or Vienna	16 oz.
		Canned roast beef or corned beef	16 oz.
		Hash, corned beef	16 oz.
		Fish, dried	14 oz.
		Fish, canned	16 oz.
		Cheese, not exceeding 10 per cent of total issue	10 oz.
Bread, soft	16 oz.	Flour, cornmeal, oatmeal or macaroni in lieu of an equal quantity bread, but not exceeding 15 per cent of total issue.	
Baking powder (To be issued only with flour or cornmeal, 1 oz. to 20 oz.)	0.08 oz.		
Beans, dry (Not to exceed 4 issues in 10 days.)	4 oz	Beans, baked (Not to exceed 4 issues in 10 days.)	8 oz
Rice or hominy (Not to exceed 6 issues in 10 days.)	2 oz.		
Potatoes, fresh	20 oz.	Onions, fresh, in lieu of an equal quantity of potatoes, but not exceeding 20 per cent of total issue. Tomatoes, canned, in lieu of an equal quantity of potatoes, but not exceeding 20 per cent. of total issue.	
		Canned potatoes	15 oz.
Potatoes, fresh	20 oz. (continued)	Other fresh vegetables (not canned) when they can be obtained in the vicinity by purchase or from U. S. Garden Service, or can be transported in a wholesome condition from a distance, in lieu of an equal quantity of potatoes.	
		Dehydrated vegetables; to be issued only in case fresh vegetables are not available	4 oz.
		Corn, canned	12 oz.
		Peas, canned	20 oz.
Jam	3 oz	Prunes, or evaporated apples, or peaches, or apricots, or figs, or dates, or raisins, in lieu of an equal quantity of jam.	
		Syrup	0.64 gil
Coffee R. & G.	1.12 oz.	Tea, black or green	0.32 oz.
Sugar	3.2 oz.		
Milk, evap., unsweetened	1. oz.		
Vinegar	0.16 gill	Pickles, cucumber, in lieu of an equal quantity of vinegar.	
Salt	.64 oz.		
Pepper, black	.02 oz.		
Cinnamon	.014 oz.	Cloves, or ginger, or nutmeg, or sage, thyme or allspice	.014 oz.
Butter	0.5 oz.	Oleomargarine or lard or lard substitutes	0.5 oz.
Flavoring extract, vanilla	.014 oz.	Flavoring extract, lemon	.014 oz.
Candy (Issued ½ lb. once in 10 days)	0.8 oz.		
Tobacco, smoking (100 cigarette papers for each 4 ounces smoking tobacco.)	0.4 oz.	Cigarettes (number)	4
		Tobacco, chewing	0.4 oz.

3. The Chief Quartermaster, A. E. F., is authorized to grant such increase in ration, or to make such modification of the ration, allowed to troops not serving in front line sectors who are engaged in hard labor for long periods as he may consider necessary; provided that the cost of the ration as increased or modified may not exceed the value of the garrison ration by more than 10 per cent and that cash savings will not be paid. The need for such additional allowances or modifications will be investigated by the Director of Laboratories, Food and Nutrition Section, Medical Department, who will report the findings with recommendations to the Chief Quartermaster.

4. The following additions are authorized to the garrison ration for troops serving in front line sectors at the discretion of division or other independent commanders, when such troops actually occupy positions in contact with the enemy:

Entire year:

	Per cent	
Bread or its substitutes	10	
Coffee	33⅓	(To be issued in the form of soluble coffee—George Washington or equal, 0.25 oz. per ration.)
Sugar	25	
Candles	100	
Solidified alcohol	1.0 oz.	(To be issued only for heating coffee or food when other fires cannot be used.)

November 1 to March 31, inclusive:

Fresh beef or its substitutes	25 per cent., or canned soup 8 oz. per ration, or soup cube one (1.76) per ration.

5. Division and other organization quartermasters are authorized and directed to purchase locally, with the consent of the local French authorities, and to issue in lieu of an equal weight of potatoes such fresh vegetables as may be obtained in good condition and at reasonable rates.

6. FIELD RATION.

COMPONENT ARTICLES AND QUANTITIES.		SUBSTITUTIVE ARTICLES AND QUANTITIES.	
*Canned meats	16 oz.	*Beef, corned, canned	16 oz.
		*Hash, corned beef, canned	16 oz.
		*Beef, fresh, roast, canned	16 oz.
		*Fish, canned	16 oz.
		*Sardines, not over 25 per cent issue	16 oz.
		*Bacon	12 oz.
*Potatoes, dehydrated	4 oz.	Potatoes, fresh	8 oz.
		*Dehydrated vegetables	4 oz
*Soup cubes, one (50 gm.)	1.76 oz.		
*Hard bread	16 oz.	Soft bread	16 oz.
*Beans, dry	4 oz.	*Peas, dry	4 oz.
*Jam	3 oz.	Dried fruits	3 oz.
*Coffee, soluble (G. W. or equal—soluble coffee to front line troops only	0.75 oz.	Coffee, R. & G.	1.12 oz.
*Sugar	3.2 oz.		
*Salt	0.16 oz.		
*Milk, evaporated	1.00 oz.		

COMPONENT ARTICLES AND QUANTITIES.		SUBSTITUTIVE ARTICLES AND QUANTITIES.	
*Chocolates, sweet	1.00 oz	Candy	1 oz.
*Tobacco, smoking (100 cigarette papers with each 4 oz. smoking tobacco.)	0.4 oz.	*Cigarettes (number)	4
		*Tobacco, chewing	0.4 oz.
*Solidified alcohol (To be issued only to troops unable to use other fires for heating food.)	2 oz.		

NOTE.—Only the articles marked * will be issued to troops in the Zone of the Armies or for stocking field and supply trains of organizations en route to the Zone of the Armies.

7. RESERVE RATION.

COMPONENT ARTICLES AND QUANTITIES.		SUBSTITUTIVE ARTICLES AND QUANTITIES.
Canned meats (Corned beef, fresh roast beef, corned beef hash in 1 lb. cans. Sardines and canned fish in 1 lb. cans or commercial tin containers to net fractions of 1 lb.)	16 oz.	
Hard bread	16 oz.	
Coffee, soluble, (G. W. or equal)	0.75 oz.	
Salt	0.16 oz.	
Sugar	3.2 oz.	
Soup, cube, one (50 gm.)	1.76 oz.	
Solidified alcohol	2.0 oz.	

(To be issued only to troops unable to use other fires for heating food.)

8. SPECIAL RESERVE RATION.

The following special reserve ration in addition to the reserve ration is authorized for troops in active operations when approved by the division or other independent commander. These rations will be packed in galvanized iron cans, hermetically sealed, each can containing 25 complete rations as follows:

50	½-lb. cans hard bread.
10	1-lb. cans corned beef.
5	1-lb. cans roast beef.
5	1-lb. cans corned beef hash.
4	1-lb. cans fish, canned.
4	¼-lb. cans sardines.
25	rations sugar in bulk (5 lbs.).
25	rations coffee, soluble (G. W. or equal) (18.75 oz).
25	rations salt in bulk.
50	oz. solidified alcohol.

9. The reserve ration and the special ration will be supplemented by any or all components of the field ration whenever possible.

10. TRAVEL RATION.

COMPONENT ARTICLES AND QUANTITIES.		SUBSTITUTIVE ARTICLES AND QUANTITIES.	
Canned roast beef	12 oz.	Canned corned beef	12 oz.
		Sardines	12 oz.
		Fish, canned	12 oz.
Soft bread	16 oz.	Hard bread	16 oz.
Beans, baked, canned	4 oz.		
Tomatoes, canned	8 oz.		
Jam	2 oz.		
Coffee R. & G.	1.12 oz.	Coffee, soluble (G. W. or equal)	0.5 oz.
Sugar	2.4 oz.		
Milk, evaporated, unsweetened	1 oz.		
Tobacco, smoking	0.4 oz.	Cigarettes (number)	4
(100 cigarette papers with each 4 oz. smoking tobacco.)		Tobacco, chewing	0.4 oz.

NOTE.—Where arrangements can be made in advance for troops to be supplied hot coffee or tea or cocoa from canteens en route, the coffee, sugar and milk components of this ration will not be issued.

III. 1. The garrison ration will be issued in kind to all troops of the A. E. F. in so far as practicable. Rations herein specified other than the garrison ration will be issued under the appropriate conditions for which designated or applicable.

2. Instructions will be given from time to time by the Chief Quartermaster, A. E. F., stating the proportions of the various substitutes and the plan of issue of the rations supplied to the troops of the A. E. F.

3. All previous orders on this subject and all instructions heretofore issued, insofar as they conflict with the above, are hereby revoked. (2873-A-30 A. G. O.)

By command of General Pershing:

JAMES W. McANDREW,
Chief of Staff.

Official:
ROBERT C. DAVIS,
Adjutant General.

ANTIPNEUMOCOCCUS PROTECTIVE SUBSTANCES IN NORMAL CHICKEN SERUM.

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ANTIPNEUMOCOCCUS PROTECTIVE SUBSTANCES IN NORMAL CHICKEN SERUM.*

BY CARROLL G. BULL, M. D., AND CLARA M. McKEE.

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The natural immunity of birds, particularly the pigeon and the domestic fowl, to infection by the pneumococcus has been a subject of research on the part of experimental immunologists since the earliest days of bacteriology. The solution of the mechanism of this phenomenon has been the chief objective, and the desire of the investigators has been to acquire a knowledge of the fundamental laws of immunity phenomena which might serve to guide them in the production and use of artificial immunity as a prophylactic and therapeutic agent.

While studying his ''maladie nouvelle'' (septicemia produced in rabbits by inoculations of sputum) Pasteur (1) discovered a new organism which, on microscopic examination, reminded him of the bacillus of chicken cholera. He inoculated chickens with the new organism and found them to be quite refractory to it. After the identity of Pasteur's sputum-septicemia organism and the pneumococcus of Fränkel was established, other workers became interested in Pasteur's original observation and it was soon found that not only chickens but pigeons and other birds are highly resistant to infection by the pneumococcus. Fränkel (2), Gamaléia (3), Tchistovitch (4) and Welch (5) were among the earliest workers who confirmed and extended Pasteur's experiments. More recently Strouse (6), Keyes (7) and Berry and Melick (8) have done interesting and important work on this subject, especially on the mechanism of the phenomenon.

Tchistovitch (4) concluded that wandering phagocytic cells play a decisive part in the pigeon's resistance to the pneumococcus. This author injected pneumococcus cultures into the anterior eye chamber of pigeons and found that the organisms did not multiply and that phagocytes soon appeared and engulfed the cocci which he had injected. Similar experiments performed on the rabbit showed that in this animal the cocci rapidly increased in number, few phagocytes appeared and a general infection developed causing death within a short time. Strouse's (6) experiments seemed to show that the normal high temperature of the pigeon is an important, if not decisive, factor in its immunity to the pneumococcus. Keyes (7) questions Strouse's conclusion and presents experiments which show that certain phagocytic cells (the hemophages) of the liver and spleen are very active in the destruction of pneumococci following intraperitoneal or intravenous injections of the organism. Keyes admits the possibility of a high temperature influence but the results of his experiments on this point tend to exclude the temperature of the pigeon as an important factor.

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That phagocytic cells finally take up and destroy pneumococci which have been injected into refractory animals (including both naturally immune and artifically immunized animals) seems to be well established. It is also well established that the sera of immunized animals contain immune bodies which can be demonstrated in vitro, and also confer passive immunity upon susceptible animals. Whether normal humoral factors play a significant or esential part in the mechanism of the resistance of naturally immune animals is, however, still debatable. Neither Strouse (6) nor Keyes (7, 9) found any evidence of antibodies in the body fluids of either the chicken or the pigeon. In testing the serum of chickens super-immunized with pneumococcus, Keyes (9) used the serum of normal chickens as a control and found that the normal serum did not protect mice against infection with the pneumococcus, while the serum of the artificially immunized chickens was very potent in this respect. Bull and Bartual (10) published experiments which showed that pneumococci are phagocyted in the fresh, undiluted blood of both chickens and pigeons. This furnished at least presumptive evidence that the blood contained opsonins for the pneumococcus since it is a general rule that washed cells of the most resistant animal are not capable of phagocyting virulent bacteria (pneumococci particularly) if they have not been previously sensitized (opsonized) with the serum of an immune animal. In a further investigation of the humoral phase of the natural immunity of the domestic fowl to pneumococci, we have made the observations presented in this paper.

EXPERIMENTAL.

Our first experimental objective was to prove the presence or absence of protective substances in normal chicken serum for any or all of the types of pneumococci. Experiments were planned with the view of affording optimal conditions for protection. Experiment A will serve to illustrate the type of protocol used for this purpose.

Experiment A.—(*a*) The serum was obtained by taking blood (20 to 40 cc.) from each of four or five large hens or roosters. The blood was collected aseptically, allowed to clot at room temperature, put in an ice box over night and the clear serum removed the next morning.

(*b*) The strains of pneumococci used in this and subsequent experiments had been passed through mice and possessed a high degree of virulence for these animals, the lethal dose being not less than 0.0000001 cc. of an eighteen-hour culture. The cultures were grown in veal infusion broth to which defibrinated rabbit blood (5 per cent. by volume) had been added.

(*c*) Mice of about 25 grams weight were given, intraperitoneally, 1 cc. of fresh chicken serum and four hours later inoculated, also intraperitoneally, with from 10 to 100,000 lethal doses of pneumococcus culture. Control mice which had received no serum were infected at the time of inoculating the test animals.

The experiment is tabulated in Protocols 1, 2, 3, 4, 5 and 6 which represent respectively infection with pneumococci of Types I, II, II A, II B, III and Group IV.

PROTOCOL 1.

Mouse protection against Type I pneumococcus with normal chicken serum.

Mice.	Serum.	Type II pneumococcus.	Results.
1......	1 cc.	0.01 cc.	D. 48 hrs.
2......	1 cc.	0.001 cc.	S. 5 days
3......	1 cc.	0.0001 cc.	S. 5 days
4......	1 cc.	0.00001 cc.	S. 5 days
5......	1 cc.	0.000001 cc.	S. 5 days
6......	—	0.0000001 cc.	D. 36 hrs.

D., dead; S., survived 5 days and discarded.

PROTOCOL 2.

Mouse protection against Type II pneumococcus with normal chicken serum.

Mice	Serum.	Type I pneumococcus.	Results.
1......	1 cc.	0.01 cc.	D. 48 hrs.
2......	1 cc.	0.001 cc.	S. 5 days
3......	1 cc.	0.0001 cc.	S. 5 days
4......	1 cc.	0.00001 cc.	S. 5 days
5......	1 cc.	0.000001 cc.	S. 5 days
6......	—	0.0000001 cc.	D. 24 hrs.

PROTOCOL 3.

Mouse protection against Type II A pneumococcus with normal chicken serum.

Mice.	Serum.	Type II A pneumococcus.	Results.
1......	1 cc.	0.01 cc.	D. 48 hrs.
2......	1 cc.	0.001 cc.	S. 5 days
3......	1 cc.	0.0001 cc.	S. 5 days
4......	1 cc.	0.00001 cc.	S. 5 days
5......	1 cc.	0.000001 cc.	S. 5 days
6......	—	0.0000001 cc.	D. 36 hrs.

PROTOCOL 4.

Mouse protection against Type II B pneumococcus with normal chicken serum.

Mice.	Serum.	Type II B pneumococcus.	Results.
1......	1 cc.	0.01 cc.	D. 48 hrs.
2......	1 cc.	0.001 cc.	S. 5 days
3......	1 cc.	0.0001 cc.	S. 5 days
4......	1 cc.	0.00001 cc.	S. 5 days
5......	1 cc.	0.000001 cc.	S. 5 days
6......	—	0.0000001 cc.	D. 36 hrs.

PROTOCOL 5.

Mouse protection against Type III pneumococcus with normal chicken serum.

Mice.	Serum.	Type III pneumococcus.	Results.
1......	1 cc.	0.0001 cc.	D. 48 hrs.
2......	1 cc.	0.00001 cc.	S. 5 days
3......	1 cc.	0.000001 cc.	S. 5 days
4......	1 cc.	0.0000001 cc.	S. 5 days
5......	—	0.0000001 cc.	D. 36 hrs.

PROTOCOL 6.

Mouse protection against Group IV pneumococcus with normal chicken serum.

Mice.	Serum.	Group IV pneumococcus.	Results.
1......	1 cc.	0.01 cc.	D. 48 hrs.
2......	1 cc.	0.001 cc.	S. 5 days
3......	1 cc.	0.0001 cc.	S. 5 days
4......	1 cc.	0.00001 cc.	S. 5 days
5......	1 cc.	0.000001 cc.	S. 5 days
6......	—	0.0000001 cc.	D. 36 hrs.

The protocols above, which were repeated a number of times, show very decisively that the serum of normal chickens contains protective substances for every type of the pneumococcus. The serum gave less protection against Type III than against the other types. In no instance did mice survive which had received more than 0.00001 cc. of culture. The 24-hour lethal dose of this culture was 0.0000001 cc.

Protection against the other types was quite regular up to 0.001 cc. of culture. Occasionally mice which had received 0.001 cc. died of infection from two to five days after being inoculated and there were sometimes irregular deaths, i.e., a mouse which had received 0.0001 cc. of culture would die while the mouse having received ten times this amount of culture survived. The most regular results were obtained in protection against our Type II culture. Time was saved by always running duplicate experiments and thus eliminating the disturbance of irregular deaths from specific infection and accident.

Experiments planned to determine the optimum conditions for protection and the nature of the protective substance gave the following results: (1) Mice manifest the highest degree of resistance when infected from eight to twelve hours after the serum has been given; there is much less protection when the serum and culture are mixed and immediately injected; twenty-four hours after the administration of the serum the mice are only slightly more resistant than normal controls. (2) If the serum is given subcutaneously, the mice do not manifest an appreciable increased resistance at any time. (3) Less than 0.5 cc. of the serum affords little protection; while 3 cc. of serum is injurious to the mice. Two cc. of serum gives more protection than 1 cc. but the difference is not great. (4) Serum from 2 to 3 weeks old is as potent as fresh serum and it is less toxic. (5) Heating the serum for thirty minutes at 56° C. does not affect its potency, while serum heated for thirty minutes at 65° C. has lost its protective power. (6) The serum of old chickens is more potent than that of young ones.

Two other points that have been determined are of sufficient interest to be given in detail: (1) The fraction of the serum in which the protective substances reside, and (2) the type specificity of the protective substances.

Fractionation of the serum.

For this work the serum was obtained by collecting blood from a large number (100 to 200) of chickens, allowing it to clot, and centrifugalizing the clot within two or three hours after collecting the blood. The serum was not sterile but there was no opportunity for bacterial growth. A type experiment is given below (Experiment B).

Experiment B.—Two liters of serum were diluted with four liters of tap water. Two liters of a saturated solution of ammonium sulphate were slowly added to the diluted serum, the mixture being constantly stirred. This stood at room temperature until the following day. A light, flocculent precipitate (precipitate A) had formed. The precipitate was collected on hardened filter paper. 3.75 liters of saturated sulphate solution were added to 7.5 liters of the filtrate obtained above. This caused a heavy precipitate to form which was filtered off the next day. This precipitate will be designated precipitate B. The filtrate obtained here was saturated with ammonium sulphate crystals and allowed to stand for 24 hours when the precipitate, which was now rising and leaving a clear and colorless fluid below, was collected on hardened filter paper. This last precipitate will be called precipitate C. The three precipitates were thoroughly pressed and then put into collodion bags and dialyzed in running tap water until they were free of the sulphate. The contents of the bags containing precipitates A and C were removed and rendered isotonic with sodium chloride cystals. Precipitate C went into solution during the dialysis and did not reprecipitate as the sulphate disappeared. Precipitate A did reprecipitate and dissolved only slightly on the addition of the sodium chloride, but almost complete solution was effected by rendering it alkaline with sodium bicarbonate. A heavy precipitate had formed in the bag containing precipitate B. A portion of the supernatant fluid was removed and rendered isotonic with sodium chloride. The precipitate and the remainder of the supernatent fluid were removed together and made isotonic. The precipitate dissolved immediately on the addition of the salt. The four solutions obtained from the dialysates were passed through Mandler filters and then tested for antipneumococcus protective substances. The first solution (''fibrinoglobulin''), it will be remembered, came from precipitate A which was formed when one fourth volume of saturated ammonium sulphate was added to the diluted serum. The second solution (''pseudoglobulin'') is the supernatant fluid from the dialysate of precipitate B (one half saturation precipitate) and is therefore largely the water soluble globulin. The third solution (''euglobulin'') is from the precipitate from dialysate B and consists principally of the water insoluble globulin. The last solution (albumin) is the dialysate from precipitate C or the precipitate which formed between 50 and 100 per cent. saturation of the serum with ammonium sulphate and consists, therefore, of the albumin fraction of the serum.

Guinea pigs weighing between 180 and 200 grams were used in testing the potency of the serum fractions which we have just described. A strain of Type II pneumococcus was used to infect the pigs. This strain had been passed through a number of guinea pigs and was very virulent for these animals, 0.000001 cc. of an 18-hour blood broth culture being the 24-hour lethal dose. Mice were also used in a few experiments and the results corresponded with those obtained with the guinea pigs. Protocols 7, 8, 9, and 10 will serve to illustrate the type of experiment employed in these tests and the results obtained. The guinea pigs were infected three hours after the serum fractions had been given, both being given intraperitoneally.

PROTOCOL 7.

Guinea pig protection against Type II pneumonococcus with the "Fibrinoglobulin" of chicken serum.

Guinea pig.	Globulin.	Type II pneumococcus.	Results.
1......	2 cc.	0.01 cc.	D. 40 hrs.
2......	2 cc.	0.001 cc.	D. 4 days
3......	2 cc.	0.0001 cc.	D. 3 days
4......	2 cc.	0.00001 cc.	D. 4 days
5......	—	0.000001 cc.	D. 40 hrs.
6......	2 cc.	—	S. 7 days

D., dead; S., survived 7 days and discarded.

PROTOCOL 8.

Guinea pig protection against Type II pneumococcus with the "Pseudoglobulin" of chicken serum.

Guinea pig.	Globulin.	Type II pneumococcus.	Results.
1......	2 cc.	0.01 cc.	S. 7 days
2......	1 cc.	0.01 cc.	D. 6 days
3......	1 cc.	0.001 cc.	S. 7 days
4......	1 cc.	0.0001 cc.	S. 7 days
5......	—	0.000001 cc.	D. 40 hrs.
6......	2 cc.	—	S. 7 days

PROTOCOL 9.

Guinea pig protection against Type II pneumococcus with the "Euglobulin" of chicken serum.

Guinea pig.	Globulin.	Type II pneumococcus.	Results.
1......	2 cc.	0.1 cc.	S. 7 days
2......	1 cc.	0.1 cc.	D. 6 days
3......	1 cc.	0.01 cc.	S. 7 days
4......	1 cc.	0.001 cc.	S. 7 days
5......	—	0.000001 cc.	D. 40 hrs.
6......	2 cc.	—	S. 7 days

PROTOCOL 10.

Guinea pig protection against Type II pneumococcus with the albumin of chicken serum.

Guinea pig.	Albumin.	Type II pneumococcus.	Results.
1......	2 cc.	0.1 cc.	D. 21 hrs.
2......	2 cc.	0.01 cc.	D. 24 hrs.
3......	2 cc.	0.001 cc.	D. 28 hrs.
4......	2 cc.	0.0001 cc.	D. 40 hrs.
5......	—	0.000001 cc.	D. 40 hrs.
6......	2 cc.	—	S. 7 days

From Protocol 7 it is seen that the precipitate formed on adding one fourth volume of saturated suluphate solution to the diluted serum contained very little of the protective substances. Two cc. of the dialysate from this precipitate did not give permanent protection. Other tests showed that the precipitate formed by 23 per cent. saturation did not contain even a trace of the protective substances. The albumin fraction did not give protection (Protocol 10). Both the water-soluble and the water-insoluble fractions gave permanent protection (Protocols 8 and 9). The water-insoluble fraction was, however, definitely more potent than the water-soluble fraction, 2 cc. of the former protecting against 0.1 cc. of culture and 1 cc. against 0.01 cc. of culture, while it took 2 cc. of the water-soluble fraction to protect against 0.01 cc. of culture. These results might be taken to indicate that the protective substances are associated with both globulin fractions but the methods used in this experiment did not insure complete separation of the two fractions. A further experiment (Experiment C) was undertaken in which a complete separation of the globulin on the basis of water solubility was attempted.

Experiment C.—The globulin was removed from the serum by salting and consisted of the precipitate which formed on one half saturation with ammonium sulphate, the one fourth saturation precipitate having been previously removed. The precipitate was dialyzed against running tap water until it was free of sulphate. The fluid portion of the dialysate was removed and dialyzed against distilled water for 24 hours. A precipitate had formed. This was removed by filtering through hardened paper and the filtrate was put into a new bag and redialyzed. This process was repeated until a 24-hours' dialysis against distilled water caused no precipitate to form. The dialysate was now rendered isotonic filtered and tested for antipneumococcus protective potency. The solid portion of the original dialysate was put into two liters of distilled water, thoroughly shaken and recollected by centrifugalization. The washing process was repeated and the sediment from the centrifugalization was dissolved in the smallest possible quantity of physiological salt solution. This solution was filtered and tested for antipneumococcus protective power. The protection tests are given in Protocols 11 and 12.

PROTOCOL 11.

Guinea pig protection against Type II pneumococcus with water-insoluble globulin.

Guinea pig.	Globulin.	Type II pneumococcus.	Results.
1......	0.1 cc.	0.1 cc.	D. 40 hrs.
2......	0.1 cc.	0.01 cc.	S. 7 days
3......	0.1 cc.	0.001 cc.	S. 7 days
4......	0.2 cc.	0.1 cc.	S. 7 days
5......	0.2 cc.	0.01 cc.	S. 7 days
6......	0.2 cc.	0.001 cc.	S. 7 days
7......	—	0.000001 cc.	D. 40 hrs.

PROTOCOL 12.

Guinea pig protection against Type II pneumococcus with water-soluble globulin.

Guinea pig.	Globulin.	Type II pneumococcus.	Results.
1......	2.4 cc.	0.1 cc.	D. 40 hrs.
2......	2.4 cc.	0.01 cc.	D. 40 hrs.
3......	2.4 cc.	0.001 cc.	D. 40 hrs.
4......	—	0.000001 cc.	D. 40 hrs.
5......	2.4 cc.	—	S. 7 days

Protocols 11 and 12 show that only the water-insoluble fraction of the globulin contains the protective substances. Two tenth of this fraction of the serum protected against 0.1 cc. of culture or 100,000 lethal doses, while 2.4 cc. of the water-soluble fraction did not protect against 0.001 cc. of culture.

TYPE SPECIFICITY OF THE PROTECTIVE SUBSTANCES.

It has been shown above that the serum of chickens protects mice and guinea pigs against any of the various types of pneumococci. Since in artificially produced immune sera the protective substances are type specific (a distinct substance for each type) and can be selectively removed by bacterial adsorption (11), it becomes of interest to know whether the chicken serum contains a distinct protective substance for each type or only one substance which has the property of protecting against any and all of the different types. The principle of selective antibody adsorption was here taken advantage of. The details of the work are given under Experiment D.

Experiment D.—(General technique.) About 150 cc. of a 24-hour broth culture of pneumococci was centrifugalized at high speed until the cocci were packed firmly on the bottom of the tubes. The supernatant culture fluid was carefully and completely removed, an effort being made to get the bacteria as dry as possible. Fresh chicken serum (30 cc. for each 150 cc. of culture) was put into the tubes containing the sedimented culture and agitated until an even suspension of the cocci had been effected. The suspension was placed in the incubator (37° C.) for two hours and then sedimented by centrifugalization. The supernatant serum was removed and passed through a Mandler filter to remove the remaining bacteria. A series of three mice for each type of pneumococcus to be used for infection was given, intraperitoneally, 1 cc. each of the adsorbed serum, and an equal number of mice was given 1 cc. of an unadsorbed portion of the same lot of serum. Four hours later all the mice were infected as indicated in the following representative protocols.

PROTOCOL 13*a*.

Mouse protection against Types I and II pneumococcus with chicken serum adsorbed with Type I pneumococcus.

Mice.	Ad. S.	N. S.	Pn. I.	Pn. II.	Results.
1....	1 cc.		0.0001 cc.		D. 24 hrs.
2....	1 cc.		0.00001 cc.		D. 24 hrs.
3....	1 cc.		0.000001 cc.		D. 30 hrs.
4....		1 cc.	0.0001 cc.		S. 5 days
5....		1 cc.	0.00001 cc.		S. 5 days
6....		1 cc.	0.000001 cc.		S. 5 days
7....	1 cc.			0.0001 cc.	S. 5 days
8....	1 cc.			0.00001 cc.	S. 5 days
9....	1 cc.			0.000001 cc.	S. 5 days
10....		1 cc.		0.0001 cc.	S. 5 days
11....		1 cc.		0.00001 cc.	S. 5 days
12....		1 cc.		0.000001 cc.	S. 5 days
13....			0.0000001 cc.		D. 30 hrs.
14....				0.0000001 cc.	D. 24 hrs.

Ad. S., Adsorbed serum.
N. S., Normal serum.
Pn. I, 18-hour blood broth culture of Type I pneumococcus.
Pn. II, 18-hour blood broth culture of Type II pneumococcus.

PROTOCOL 13*b*.

Mouse protection against Types I and II pneumococcus with chicken serum adsorbed with Type II pneumococcus.

Mice.	Ad. S.	N. S.	Pn. I.	Pn. II.	Results.
1....	1 cc.		0.0001 cc.		S. 5 days
2....	1 c.c		0.00001 cc.		S. 5 days
3....	1 cc.		0.000001 cc.		S. 5 days
4....		1 cc.	0.0001 cc.		S. 5 days
5....		1 cc.	0.00001 cc.		S. 5 days
6....		1 cc.	0.000001 cc.		S. 5 days
7....	1 cc.			0.0001 cc.	D. 24 hrs.
8....	1 cc.			0.00001 cc.	D. 24 hrs.
9....	1 cc.			0.000001 cc.	D. 24 hrs.
10....		1 cc.		0.0001 cc.	S. 5 days
11....		1 cc.		0.00001 cc.	S. 5 days
12....		1 cc.		0.000001 cc.	S. 5 days
13....			0.0000001 cc.		D. 36 hrs.
14....				0.0000001 cc.	D. 24 hrs.

From Protocols 13 *a* and 13 *b* it is seen that serum adsorbed with Type I pneumococci no longer protects mice against this type but still protects against Type II as well as the unadsorbed serum, and that adsorption with Type II removes the protective substance for this type without removing the Type I protective substance. It appears, therefore, that normal chicken serum contains distinct protective substances for these two types of pneumococci.

Other experiments, similar to Experiment D, showed that chicken serum contains a specific protective substance for each type of pneumococcus. Only one virulent strain of Group IV was available but serum adsorbed with this strain no longer protected against itself but still protected against Types I, II and III, and adsorption with any of these types did not remove the protective substance for the Group IV strain.

It now became of interest to know whether the type specificity of chicken serum extended to the subtypes of Type II strains. These subtypes, it will be remembered, consist of atypical Type II organisms or strains of pneumococci which are agglutinated only slowly and incompletely with Type II serum. In a study of these atypical Type II strains Avery (11) discovered two new serological types which he designated Type II A and Type II B. The relation between these new types and Type II was as follows: A Type II immune serum contains agglutinins and protective substances for all three types but an immune serum produced with a strain of either of the other types (II A or II B) contains antibodies only active against the homologous type. A Type II serum adsorbed with a Type II culture no longer protects against or agglutinates Type II, II A or II B strains, all the antibodies being removed. If a Type II serum is adsorbed with either II A or II B organisms only the homologous antibodies are removed, and the antibodies are not removed from a Type II A immune by adsorption with a II B culture and vice versa. It is seen, therefore, that a Type II immune serum contains one group of major antibodies and two groups of minor antibodies, the latter groups being distinct from each other or, in other words, Types II A and II B are subgroups of Type II.

Avery's experiments were repeated, using chicken serum instead of the sera of immunized animals. Our work is given in detail under Experiment E.

*Experiment E.**—Three series of mice were given, intraperitoneally, 1 cc. of chicken serum which had been adsorbed (see Experiment D) with either a Type II, II A or II B culture and were infected four hours later—one series with Type II culture, one with Type II A and one with Type II B. The three following protocols give the different experiments in tabular form.

PROTOCOL 14.

Mouse protection against Types II, II A and II B pneumococcus with chicken serum adsorbed with Type II pneumococcus.

Mice.	Ad. S.*	Pn. II.	Pn. II A.	Pn. II A.	Results.
1....	1 cc.	0.0001 cc.			D. 24 hrs.
2....	1 cc.	0.00001 cc.			D. 24 hrs.
3....	1 cc.	0.000001 cc.			D. 24 hrs.
4....	1 cc.		0.0001 cc.		D. 3 days
5....	1 cc.		0.00001 cc.		S. 5 days
6....	1 cc.		0.000001 cc.		S. 5 days
7....	1 cc.			0.0001 cc.	D. 2 days
8....	1 cc.			0.00001 cc.	S. 5 days
9....	1 cc.			0.000001 cc.	S. 5 days
10....		0.0000001 cc.			D. 24 hrs.
11....			0.0000001 cc.		D. 48 hrs.
12....				0.0000001 cc.	D. 48 hrs.

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* The strains of Types II A and II B pneumococci used in this experiment were kindly furnished us by Dr. O. T. Avery, of the Hospital of the Rockefeller Institute.

* The same number of mice was given 1 cc. each of an unadsorbed portion of the same lot of serum and infected in the same way and at the same time as the

PROTOCOL 15.

Mouse protection against Types II, II A and II B pneumococcus with chicken serum adsorbed with Type II A pneumococcus.

Mice.	Ad. S.	Pn. II.	Pn. II A.	Pn. II B.	Results.
1....	1 cc.	0.0001 cc.			D. 24 hrs.
2....	1 cc.	0.00001 cc.			D. 24 hrs.
3....	1 cc.	0.000001 cc.			D. 24 hrs.
4....	1 cc.		0.0001 cc.		D. 24 hrs.
5....	1 cc.		0.00001 cc.		D. 24 hrs.
6....	1 cc.		0.000001 cc.		D. 48 hrs.
7....	1 cc.			0.0001 cc.	S. 5 days
8....	1 cc.			0.00001 cc.	S. 5 days
9....	1 cc.			0.000001 cc.	S. 5 days
10....		0.0000001 cc.			D. 24 hrs.
11....			0.0000001 cc.		D. 48 hrs.
12....				0.0000001 cc.	D. 48 hrs.

PROTOCOL 16.

Mouse protection against Types II, II A and II B pneumococcus with chicken serum adsorbed with Type II B pneumococcus.

Mice.	Ad. S.	Pn. II.	Pn. II A.	Pn. II B.	Results.
1....	1 cc.	0.0001 cc.			D. 24 hrs.
2....	1 cc.	0.00001 cc.			D. 24 hrs.
3....	1 cc.	0.000001 cc.			D. 24 hrs.
4....	1 cc.		0.0001 cc.		S. 5 days
5....	1 cc.		0.00001 cc.		S. 5 days
6....	1 cc.		0.000001 cc.		S. 5 days
7....	1 cc.			0.0001 cc.	D. 24 hrs.
8....	1 cc.			0.00001 cc.	D. 24 hrs.
9....	1 cc.			0.000001 cc.	D. 48 hrs.
10....		0.0000001 cc.			D. 24 hrs.
11....			0.0000001 cc.		D. 48 hrs.
12....				0.0000001 cc.	D. 48 hrs.

The protocols above show, (1) that chicken serum adsorbed with Type II pneumococci loses only the Type II protective substances, still protecting against Type II A and II B strains; (2) that serum adsorbed with Type II A culture looses the Type II A and Type II antibodies but not the Type II B antibodies; and (3) that serum adsorbed with Type II B culture looses the Type II B and Type II protective substances but not those for Type II A.

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mice which received the adsorbed serum in order to make sure that the serum contained protective substances for all three types before it was adsorbed. The same was done in Protocols 15 and 16.

In terms of the protective substances of chicken serum, therefore, Types II A and II B pneumococci seem to form two distinct main groups with Type II a subgroup to both of them. This relationship is just the reverse of that which Avery found in terms of sera from specifically immunized animals.

DISCUSSION.

The observations recorded above, in conjunction with previous observations, afford ground for concluding that specific humoral elements are essential factors of the natural immunity of the chicken to infection by the pneumococcus. The exact nature of these humoral elements or the manner in which they operate to effect resistance has not been determined. It is probable, however, that they are of the nature of opsonins and prepare the pneumococci for phagocytic destruction. The basis of these conclusions is briefly as follows: (1) The serum of chickens has the power of conferring upon other animals an immunity to the pneumococcus, (2) virulent pneumococci are phagocyted in fresh chicken blood in vitro, (3) pneumococci grow readily in fresh chicken serum or plasma, and (4) opsonization and phagocytosis are known to be essential factors in other instances of acquired and artificial immunity to the pneumococcus.

It may be objected that the passive immunity conferred upon other animals with chicken serum is not comparable in degree to that of the chicken and that the methods employed fail to demonstrate pneumococcus opsonins in chicken serum. It is to be remembered, on the one hand, that it is not necessary to have a high concentration of antibodies in the body tissues and fluids of an animal in order to have protection against infection, provided the antibodies are constantly present and are inexhaustible. On the other hand, the usual methods of studying antibodies in vitro entail a dilution of the serum and a one-fold dilution may suffice to inhibit their action. These conditions seem to obtain in the present case. The production of antipneumococcus protective substances is, no doubt, a natural function of the tissues of the chicken and whenever the normal concentration of these substances in the blood is reduced the tissues immediately liberate more of them and reestablish the equilibrium, the resistance of the animal being thus maintained. As already stated, pneumococci are phagocyted in the undiluted fresh blood of the chicken while in a mixture of one part serum, one part bacteria and one part leucocytes no phagocytosis occurs. The demonstration by Keyes (7) that phagocytosis plays a prominent rôle in the destruction of pneumococci in the pigeon is another point in favor of this explanation of the chicken's immunity to the pneumococcus. In all probability similar mechanisms operate in both instances, but as yet the humoral phase in the former and phagocytic in the latter have not been satisfactorily demonstrated.

Why chicken serum protects mice against infection with the pneumococcus when it is given intraperitoneally and fails to protect when it is given subcutaneously is not entirely clear. There are two facts, however, which afford a possible explanation for this anomaly. First, animals are more resistant to infection several hours after they have received antibacterial sera than they are if they are infected at the time of administering the serum. This is true notwithstanding the fact that the infecting organisms may be exposed to the action of the concentrated serum when the culture and the serum are given at the same time and not when the serum is given several hours before the animals are infected. It appears, therefore, that some kind of reaction between the immune serum and the tissues of the host must take place before the protective serum becomes effective. This reaction may be nothing more than the adsorption of the protective bodies by the cells of the host or it may be a more specific reaction between factors from the host and from the immune serum resulting in the formation of a new substance to which protection is due. In any case, time elapses between the administration of the serum and the development of the maximum resistance on the part of the host. The second fact is that the chicken serum is rapidly destroyed or eliminated by mice for, on intraperitoneal injection, the mice are not protected for more than twenty-four hours. Horse serum is not so quickly eliminated since antipneumococcis horse serum protects mice for at least six days. It is possible, therefore, that on subcutaneous injection the chicken serum is destroyed before the reaction necessary for protection occurs and, for this reason, a resistant state never develops.

The discovery of antibodies in the serum of a normal animal which are specific for the different serological types of the pneumococcus shows that the classification of these organisms, although done with artificially prepared sera, has a fundamental biological significance. If this classification had first been done by means of the protective substances of chicken serum, it would have been just as it is with the exception of the relation of the group II subtypes to the main type. This difference is due, doubtless, to differences in the antigenic origin of the two sets of antibodies. The observation is of interest in connection with existing theories of the origin and relationships of major and minor antibodies.

How the chicken came to possess these antipneumococcic substances is quite a puzzle to the immunologist. Existing theories concerning the origin of natural bacterial antibodies do not offer a satisfactory explanation in this instance. Is it possible that these substances owe their origin to a specific antigenic stimulation which occurred in some earlier period of the life history of the species, the germplasm being so fundamentally impressed that an acquired property became inheritable? If specific antigenic stimulation is eliminated, it gives reason for questioning the soundness of this theory with reference to other natural antibodies found in both man and the lower animals.

SUMMARY.

1. It has been shown that the serum of normal chickens is capable of protecting mice and guinea pigs against infection with pneumococci.

2. The protective substances are found in the water-insoluble fraction of the serum globulin.

3. There are, in chicken serum, particular protective substances for each serological type of pneumococcus. These substances are selectively removed from the serum by the process of bacterial adsorption.

4. In terms of the protective substances in chicken serum, Types II A and II B pneumococci constitute two distinct main groups and Type II strains form a subgroup to both of them.

REFERENCES.

1. PASTEUR, L.
1881. Note sur la maladie nouvelle provoquée par la salive d'un enfant mort de la rage. Bulletin de l'Académie de medecine, X, 94.
2 FRÄNKEL, A.
1886. Bakteriologische Mittheilungen, Erster Theil. Zeit. f. klin. Med., X, 401.
3. GAMALÉIA, M. N.
1888. Sur la étiologie de la pneumonie fibrineuse chez l'homme. Ann. de l'Inst. Pasteur, II, 440.
4. TCHISTOVITCH, N.
1890. Études sur la pneumonie fibrineuse. Ann. de l'Inst. Pasteur, IV, 285.
5. WELCH, W. H.
1892. The Micrococcus lanceolatus, with especial reference to the etiology of acute lobar pneumonia. Bull. Johns Hopkins Hospital, III, 125.

6. Strouse, S.
1909. Experimental studies on pneumococcus infections. J. Exper. Med., II, 743.

7. Keyes, P.
1916. The natural resistance of the pigeon to the pneumococcus. J. Infect. Dis., XVIII, 277.

8. Berry, F. and Melick, C. O.
1916. The site and rate of destruction of pneumococci following intraperitoneal injection. J. Immunol., I, 119.

9. Keyes, P.
1911. Production of antibodies to pneumococci in an insusceptible host. Jour. Am. Med. Ass., LVI, 1878.

10. Bull, C. G. and Bartual, L.
1920. Pneumococcus cultures in whole fresh blood. Jour. Exper. Med., XXXI, 233.

11. Avery, O. T.
1915. A further study on the biologic classification of pneumococci. Jour. Exper. Med., XXII, 804.

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A STUDY OF THE NATURAL AND ACQUIRED ANTISHEEP HEMOLYSINS OF THE RABBIT AS REGARDS THERMOLABILITY.*

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The so-called natural antibodies found in the sera of many animal species have attracted attention from various points of view, viz., their origin, specificity, relation to natural immunity and particularly their relation to the corresponding acquired antibodies. The point mentioned last is of especial significance in connection with the origin of acquired antibodies and has, therefore, been the subject of a number of investigations. The view is held by some investigators (1) (2) that the identity of these two classes of antibodies has been established, while others (3) do not accept the evidence which has been advanced in favor of their identity, or have pointed out instances wherein a natural antibody is qualitatively different from the corresponding acquired antibody. Thiele and Embleton (4) have recently advanced a theory of the origin of acquired hemolysins which involves the existence of thermolabile and thermostabile hemolysins. Sherman (5) questions the soundness of this theory because he found all hemolysins to be thermostabile. Kolmer (6), on the other hand, concludes that natural hemolysins are less resistant to heat than immune hemolysins.

The purpose of the present paper is to record observations which we have made concerning the effects of different degrees of heat on the natural and immune hemolysins of the rabbit for sheep corpuscles.

Experimental.

Four rabbits (two normal and two immune) were selected for the study on the basis of the hemolytic titers of their sera, a wide range of titer being sought. The serum of one of the normal rabbits had a titer 1–10, the other 1–100; while the serum of one of the immunized rabbits had a titer of 1–2,500 and the other 1–25,000. The point to be determined was the rate of loss of amboceptor content of the different sera heated at different temperatures.

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Technique.—The corpuscles used in the titrations were collected from the sheep in a solution of sodium citrate and then washed four times in large volumes of salt solution (0.85 per cent. NaCl) and resuspended in salt solution so that the corpuscles were one fourth as concentrated as in the original blood. One tenth of a cubic centimeter of this suspension was taken as a unit of cells. Pooled guinea pig serum was diluted with four parts of salt solution and 0.1 cc. of the diluted serum was used as a unit of complement. The sera to be titrated were heated in a water bath for thirty minutes at varying degrees of temperature (ranging from 56° C. to 84° C.) and then diluted with salt solution in order that graded quantities could be conveniently put in a series of serological tubes. Salt solution was now added to make the volume in each tube 0.8 cc. A unit of cells was then put in each tube and the tubes were placed in a water bath (temperature 37° C.) for one hour. At the end of the incubation period a unit of complement was added and the tubes were returned to the water bath for another hour. The extent of hemolysis was observed at the expiration of the second incubation period and again after the tubes had stood at room temperature for twelve hours. Different degrees of hemolysis are indicated in the protocols by numerals; 4 indicates complete hemolysis, 0 no hemolysis and the intervening figures a corresponding degree of hemolysis. Protocol 1 illustrates the type of titration experiments used throughout the work.

PROTOCOL 1.

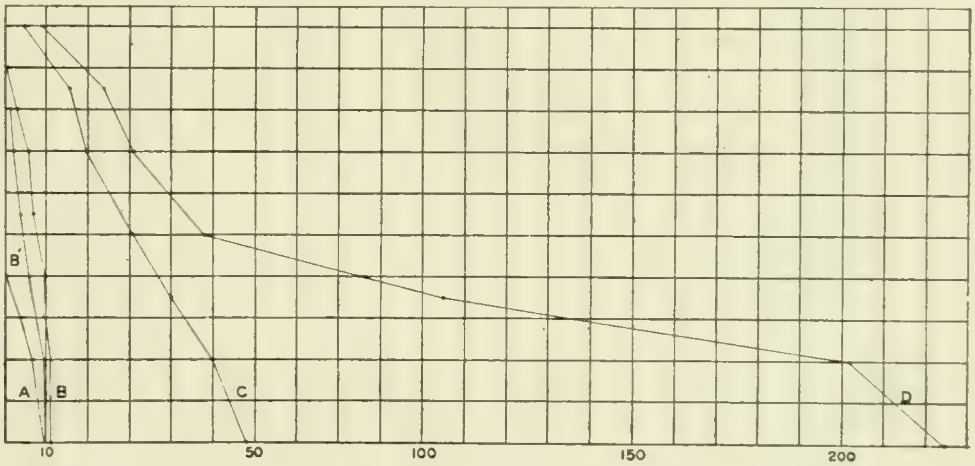
Type protocol used in the titration experiments.

Tubes.....	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	(1–0)*				(1–10)					(1–100)					
Serum...	0.6	0.4	0.2	0.1	0.6	0.4	0.3	0.2	0.1	0.8	0.6	0.4	0.2	0.0	0.0
Salt.....	0.2	0.4	0.6	0.7	0.2	0.4	0.5	0.6	0.7	0.0	0.2	0.4	0.6	0.8	0.9
Cells 1–4	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Incubated 1 hour at 37° C.															
Complement 1–5	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0

* The numbers in parenthesis indicate the extent to which the serum was diluted before the different quantities were measured into the various tubes. Thus tubes 1 to 4 contained the indicated quantities of undiluted serum and tubes 5 to 9 serum diluted 1–10 with salt solution, etc.

The effect of different temperatures on immune hemolysins.—The question here was the determination of the rate at which the immune amboceptor disappears from the serum when subjected to heat for 30 minutes through a graded series of temperatures. The two immune sera which have already been mentioned were used for this purpose. The sera are further described under sections (*a*) and (*b*), and the results of the experiment are recorded in Tables I and II respectively.

(*a*) *Antisheep rabbit 266.*—The serum from this rabbit was very potent, the titer being 1–26,000 after inactivation at 56° C. for 30 minutes. Specimens of the serum were kept at six different degrees of temperature (56° C.–84° C.) for 30 minutes and then titrated for hemolytic power. The results are collected in Table I and are represented graphically by Curve *D* in the text figure.



These curves are based on "1" degree of hemolysis at the 12-hour readings. The temperatures at which the sera were heated are expressed by the ordinates, while the abscissae express the square roots of the hemolytic titers. The curves show the rate of destruction of the hemolysins when heated through a range of temperatures above 56°C. Curve *A* represents normal rabbit serum, low titer (Table III); Curve *B*, normal rabbit serum, high titer (Table IV); Curve B,[1] immune hemolysins in normal rabbit serum (Table V); Curve *C*, immune serum, low titer (Table II); Curve *D*, immune serum, high titer (Table I).

(*b*) *Antisheep rabbit 260.*—The serum from this rabbit was about one tenth as potent as the serum from rabbit 266. The results obtained with this serum are given in Table II and Curve *C* (*see text figure*).

Curves *C* and *D* obtained with the two immune sera of widely different titers are directly comparable. It is seen that the curves are parallel beyond 66° C. and that the hemolysins practically disappear at 76° C. Between 60° C. and 66° C. there is a marked difference showing that the rate of destruction is greatest between these two temperatures for the serum of the higher titer.

TABLE I.

The effect of different temperatures on immune serum, rabbit 266.

Temperature of inactivation.	Titer of the serum at 1 hour.	Titer of the serum at 12 hours.
56° C.	35,000†	50,000
60° C.	30,000	41,000
63° C.	8,000	11,000
66° C.	1,700	2,300
70° C. (1–2)*	730	1,015
73° C. (1–2)	400	600
76° C. (1–2)	50	70
84° C. (1–4)	6	0

TABLE II.

The effect of different temperatures on immune serum, rabbit 260.

Temperature of inactivation.	Titer of the serum at 1 hour.	Titer of the serum at 12 hours.
Unheated	5,000†	6,700
56° C.	3,300	3,300
60° C.	2,200	2,500
63° C.	1,540	1,666
66° C.	1,000	1,000
70° C. (1–2)*	333	400
73° C. (1–2)	250	260
76° C. (1–2)	10	20
80° C. (1–4)	8	13

The effect of different temperatures on natural hemolysins.

(*a*) *Normal rabbit serum, low titer.*—After inactivation at 56° C. this serum failed to cause complete hemolysis in dilution above 1–10. The effect of higher degrees of temperature on this serum is shown in Table II. It is seen that the hemolysins were completely destroyed at 64° C. Curve *A* (see text figure) gives a graphic representation of the rate at which the hemolysins were destroyed.

(*b*) *Normal rabbit serum high titer.*—This serum caused complete hemolysis in a dilution of 1–50 after inactivation at 56° C. The effects of higher temperatures are shown in Table IV. It is seen that a temperature of 70° C. does not completely destroy the hemolysins of this serum (see Curve *B*).

A study of the foregoing tables shows that all the sera, both normal and immune, contained thermostabile hemolysins, i.e., hemolysins that were not destroyed by being heated for 30 minutes at 56° C. It is

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* The figures in parentheses indicate the dilution of the serum before heating.

† These numbers represent the number of hemolytic units per cc. of serum.

TABLE III.

The effect of different temperatures on natural hemolysins, serum of low titer.

Tubes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Serum	(1–0)* 0.6	0.4	0.3	0.1	(1–10) 0.6	0.5	0.4	0.3	0.2	0.1	(1–100) 0.6	0.5	0.4	0.3	0.2	0
1 hour reading:																
Active, no complement	4	4	4	1–	0	0	0	0	0	0	0	0	0	0	0	0
Unheated	4	4	4	4	1	1–	1–	1–	0	0	0	0	0	0	0	0
56° C.†	4	4	4	4	1	1–	1–	1–	1–	0	0	0	0	0	0	0
60° C.	3	3	3	3	1–	1–	1–	1–	0	0	0	0	0	0	0	0
62° C.	3	3	3	1	1–	1–	0	0	0	0	0	0	0	0	0	0
64° C.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12 hour reading:																
Active, no complement	4	4	4	2	1–	0	0	0	0	0	0	0	0	0	0	0
Unheated	4	4	4	4	2	2	2	2	1	1	1–	0	0	0	0	0
56° C.	4	4	4	4	2	2	2	2	1	1	1–	0	0	0	0	0
60° C.	3	3	3	3	3	3	3	1	1	1–	0	0	0	0	0	0
62° C.	3	3	3	3	1	0	0	0	0	0	0	0	0	0	0	0
64° C.	1–	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* The figures in parentheses indicate the extent to which the serum was diluted before the quantities indicated were added to the tubes. Thus, tubes 1–4 received undiluted serum and tubes 5–10 received serum diluted 1–10 with salt solution, etc.

† The heated serum was reactivated with a unit of complement, 0.1 cc. of guinea pig serum diluted 1–5 with salt solution.

TABLE IV.

The effect of different temperatures on natural hemolysins, serum of high titer.

Tubes	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Serum	(1–0) 0.6	0.4	0.2	0.1	(1–10) 0.6	0.4	0.3	0.2	0.1	(1–100) 0.8	0.6	0.4	0.2	0
1 hour reading:														
Active, no complement	4	4	4	4	4	3	3	1–	1–	0	0	0	0	0
Unheated	4	4	4	4	4	4	4	1–	1–	0	0	0	0	0
56° C.	4	4	4	4	4	4	4	1–	1–	0	0	0	0	0
60° C.	4	4	4	4	4	4	3	3	0	0	0	0	0	0
62° C.	3	3	3	4	4	4	3	2	0	0	0	0	0	0
64° C.	3	3	3	4	4	4	4	1	0	0	0	0	0	0
67° C.	4	4	4	4	4	3	1	0	0	0	0	0	0	0
70° C. (1–2)	3	3	3	3	2	1–	0	0	0	0	0	0	0	0
72° C. (1–2)	1	1	1	1	0	0	0	0	0	0	0	0	0	0
12 hour reading:														
Active, no complement	4	4	4	4	4	3	3	1–	1–	0	0	0	0	0
Unheated	4	4	4	4	4	4	4	4	3	2	1–	0	0	0
56 C.	4	4	4	4	4	4	3	3	3	1	1–	0	0	0
60° C.	4	4	4	4	4	4	4	4	2	1	1–	0	0	0
62° C.	3	3	3	4	4	4	4	3	1	1–	0	0	0	0
64° C.	3	3	3	4	4	4	4	3	1	1–	0	0	0	0
67° C.	4	4	4	4	4	4	3	3	1–	1–	0	0	0	0
70° C. (1–2)	4	4	4	4	4	4	4	2	1–	0	0	0	0	0
72° C. (1–2)	1	1	1	1	0	0	0	0	0	0	0	0	0	0

seen that in one of the normal sera (the one of low titer, Curve *A* the hemolysins were completely destroyed by a temperature of 64° C., while complete destruction of the hemolysins was not effected in the other normal serum (the one of high titer, Curve *B*) until a temperature of 74° C. was reached. The two immune sera, being of widely different titers, show that the number of units of hemolysin destroyed within certain temperature limits (60° C.–66° C.) depends upon the concentration of the antibody in the serum (Curves *C* and *D*), but that in each case a certain residue of antibody remains which requires practically the same amount of heating to destroy completely. A comparison of the four curves indicates that the thermal death point of the sera (complete destruction of the hemolysins) is a function of the hemolytic potency of the sera rather than the kind (natural or immune) of hemolysin present. This point will be referred to again.

The effect of heat on diluted immune hemolysins.—The foregoing experiments having indicated that the degree of heat necessary to effect complete destruction of the hemolysins in a given serum depends upon the concentration rather than the kind (natural or immune) of hemolysin present, it was desirable to test this point further by testing the effect of heat on two sera of the same potency, one containing natural hemolysins and the other immune hemolysins. For this purpose an artificial immune serum was prepared in such a way that it would have the same titer as a given normal serum. The normal serum, which has already been discussed (Table IV), contained 100 hemolytic units per cc. The immune serum was prepared by two methods and designated Solutions *A* and *B* respectively.

Solution A.—Normal rabbit serum was freshly collected and rendered free of hemolysins by adsorption at 4° C. with an excess of washed sheep corpuscles. The corpuscles were removed from the serum by centrifugalization and the desired amount of a highly potent immune serum was added. The artificial serum had a titer of 1–100 after inactivation at 56° C. for 30 minutes.

Solution B.—This preparation was the same as Solution *A* with the exception that salt solution instead of normal rabbit serum was used to dilute—the immune serum. The titer was the same as that of Solution *A*.

The effect of different degrees of heat on the hemolytic titers of both of these solutions was determined, the plan of the experiment being the same as in previous experiments. The results are given in Tables V and VI respectively.

TABLE V.

The effect of different temperatures on immune hemolysins, artificial serum A.

Tubes	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Solution A	(1–0) 0.6	0.4	0.2	0.1	(1–10) 0.6	0.4	0.3	0.2	0.1	(1–100) 0.8	0.6	0.4	0.2	0
1 hour reading:														
Adsorbed control	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unheated	4	4	4	4	4	4	4	2	2	1—	0	0	0	0
56° C.	3	2	2	2	1—	1—	1—	1—	0	0	0	0	0	0
60° C.	3	3	3	3	1—	0	0	0	0	0	0	0	0	0
62° C.	4	4	4	3	1—	0	0	0	0	0	0	0	0	0
64° C.	3	3	3	1—	1—	0	0	0	0	0	0	0	0	0
67° C.	3	3	1	1—	1—	0	0	0	0	0	0	0	0	0
70° C. (1–2)	4	3	0	0	0	0	0	0	0	0	0	0	0	0
74° C. (1–2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12 hour reading:														
Adsorbed control	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unheated	4	4	4	4	4	4	4	3	3	2	1—	0	0	0
56° C.	3	3	3	4	3	3	2	1	1—	1—	0	0	0	0
60° C.	4	4	4	4	3	3	3	2	1—	1—	0	0	0	0
62° C.	4	4	4	4	3	1	1—	0	0	0	0	0	0	0
64° C.	3	3	3	4	4	1	0	0	0	0	0	0	0	0
67° C.	4	4	3	2	1	1—	0	0	0	0	0	0	0	0
70° C. (1–2)	4	4	1	0	0	0	0	0	0	0	0	0	0	0
74° C. (1–2)	1	1—	0	0	0	0	0	0	0	0	0	0	0	0

TABLE VI.

The effect of different temperatures on immune hemolysins, artificial serum B.

Tube	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Solution B	(1-) 0.6	0.4	0.2	0.1	(1-10) 0.6	0.4	0.3	0.2	0.1	1-100 0.8	0.6	0.4	0.2	0
1 hour reading:														
56° C.	4	4	4	4	4	4	4	3	1 –	0	0	0	0	0
60° C.	4	4	4	4	4	4	4	3	1 –	0	0	0	0	0
62° C.	3	3	4	4	4	3	1	1	0	0	0	0	0	0
64° C.	3	3	3	3	3	1	0	0	0	0	0	0	0	0
67° C.	3	3	4	4	1 –	0	0	0	0	0	0	0	0	0
70° C. (1-2)	4	4	1 –	0	0	0	0	0	0	0	0	0	0	0
74° C. (1-2)	4	1 –	0	0	0	0	0	0	0	0	0	0	0	0
12 hour reading:														
56° C.	4	4	4	4	4	4	3	3	1	1 –	0	0	0	0
60° C.	4	4	4	4	4	4	4	3	2	1	0	0	0	0
62° C.	4	4	4	4	4	4	3	2	1 –	0	0	0	0	0
64° C.	3	3	3	4	4	1	1	1	0	0	0	0	0	0
67° C.	4	4	4	4	4	1	1 –	0	0	0	0	0	0	0
70° C. (1-2)	4	4	4	1	1 –	0	0	0	0	0	0	0	0	0
74° C. (1-2)	4	4	1	0	0	0	0	0	0	0	0	0	0	0

It is seen from Tables V and VI that immune hemolysins diluted with salt solution are only slightly more susceptible to the action of heat than when diluted with normal serum. It is of particular interest to note that the normal hemolytic serum (Table IV) and the artificial immune serum resist destruction by heat through practically the same range of temperatures (compare Curves *B* and *B'* in the text figure). It is further indicated therefore, that the rate at which hemolysins are destroyed by heat, as well as the temperature necessary to destroy completely the amboceptor content of a given serum, depends upon the quantity rather than the quality of the hemolysins.

TABLE VII.

The effect of salt solution on the thermolability of immune hemolysins.

Diluent.	Temperature.	1 hour, 4.	12 hours, 4.
Salt	56° C.	25,000	33,000
Serum	56° C.	25,000	33,000
Salt	60° C.	25,000	25,000
Serum	60° C.	25,000	25,000
Salt	63° C.	8,333	8,333
Serum	63° C.	8,333	8,333
Salt	66° C.	625	625
Serum	66° C.	625	625
Salt	70 C.	500	500
Serum	70° C.	500	500

The relative thermolability of hemolysins in the presence and absence of other serum constituents.—In the foregoing experiments, as will have been seen, it was necessary to dilute the hemolytic sera before testing the effects of the higher temperatures on them because undiluted serum forms a firm coagulum at these temperatures. Physiological salt solution was used as the diluent. The propriety of this procedure was questioned at the time. A casual experiment showed, however, that there was no great difference in the heat susceptibility of hemolysins when diluted with salt solution and when in undiluted serum. Since this result was not in agreement with what one would be inclined to conclude *a priori* and since the conclusions to be drawn from other experiments (see Table VI) are based upon the assumption that dilution with salt solution does not affect the thermolability of immune hemolysins, it was deemed advisable to subject this point to more exact tests.

An immune antisheep rabbit serum of very high titer was chosen for this experiment. Two preparations were made with the immune serum: (1) 1 cc. of the serum was added to 49 cc. of salt solution; (2) 1 cc. of the hemolytic serum was added to 49 cc. of normal rabbit serum from which the natural hemolysins had been removed by specific adsorption. The two preparations were heated in 3 cc. quantities at different temperatures for 30 minutes and the hemolytic titers determined. The results are given in Table VII.

Table VII shows that the thermolability of hemolysins is in no way affected by the presence or absence of other serum constituents. The first preparation contained 98 per cent. of salt solution during the heating process, while in the second preparation the salt solution was replaced by normal rabbit serum. The hemolytic titers of the preparations were exactly the same.

Discussion.

The results of the experiments recorded in this paper seem to support the contention that natural hemolysins are qualitatively the same as the corresponding acquired or immune hemolysins. It has been shown that two specimens of normal serum containing different quantities of hemolysins for a particular corpuscle will, on being heated at different temperatures, give the impression that the antibodies of one specimen are more sensitive to heat than those of the other. However, the serum containing the more sensitive antibodies is found to be the one which had the lowest original titer. It would follow, therefore, that a serum of very low titer might be entirely inactive (not contain an appreciable amount of antibody) after inactivation at 56° C. for 30 minutes, and thus give rise to the impression that only thermolabile antibodies were present, while a more potent serum of the same kind would give the opposite result. A consideration of normal sera alone leads to the conclusion that the amount of heat necessary permanently to inactivate a serum depends in part upon the quantity of antibody present.

In comparing normal and immune sera, it was found that natural and acquired hemolysins are equally resistant to heat. The comparison was made possible by obtaining normal and immune sera of the same potency. The immune serum was prepared artificially, but it was shown by experiment that its sensitivity to heat was not thus affected. It seems fair to conclude, therefore, that the apparent relative thermolability of the natural hemolysins, as compared with acquired hemolysins, is due to a quantitative rather than a qualitative difference.

SUMMARY.

1. The natural hemolysins of the rabbit for sheep corpuscles are as heat resistant as the corresponding immune hemolysins.

2. The apparent difference in the thermolability of natural and immune hemolysins is due to the relative potency of the sera, the more potent serum apparently being more resistant to heat.

3. Hemolysins are just as resistant to heat when in salt solution as in undiluted serum.

LITERATURE.

1. Ford, W. W.
1902. Beitrag zur Lehre von den Hämagglutininen. Ztschr. f. Hyg., Leipzig, XL, 363.
2. Wassermann, A.
1903. Ueber Agglutinine und Präcipitine. Ztschr. f. Hyg., Leipzig, XLII, 267.
3. Bordet, J.
1904. Les propriétés des antisensibilisatrices et les théories chimiques de l'immunité. Ann. de l'Institut Pasteur, XVIII, 593.
4. Thiele, F. H. and Embleton, Dennis.
1914. The evolution of the antibody. Ztschr. f. Immunitätsforsch. u. exper. Therap., XX, 1.
5. Sherman, H.
1918. Thermostabile and so-called thermolabile hemolysins. Jour. Infect. Dis., XXII, 534.
6. Kolmer, J. A.
1919. The nature of thermolabile hemolysins. Jour. Immunol., IV, 403.

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THE REACTIVATION OF THE NATURAL HEMOLYTIC ANTIBODY IN CHICKEN SERUM.*

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The fresh blood serum from many animal species hemolyzes the red cells of other species. In general the reaction has been shown to be due to a sensitizer-alexin complex, the natural sensitizers (hemolysins) being very similar to the specific immune hemolysins. For example, they resist heating for 30 minutes at 56° C., they sensitize red blood corpuscles to the action of alexin from other species, and they can be removed from the serum by adsorption with the red cells for which they are hemolytic. The natural hemolysins of the domestic fowl are, however, peculiar in that it has been found difficult or impossible to reactivate the serum with the fresh sera of other species after it has been rendered inactive by heat. In fact the presence of a heat resistant antibody has been questioned.

Bordet (1) was the first to observe the hemolytic properties of chicken serum. He called attention to the marked susceptibility of rabbit corpuscles to the hemolytic and agglutinating action of this serum. Metchnikoff (2) states that Bordet found it impossible to reactivate chicken serum with any other serum, after the former had been heated for 30 minutes at 55–56° C., and that he (Bordet) was of the opinion that in this instance alexin alone was capable of producing hemolysis, previous sensitization with an immune body being unnecessary. We have been unable to find the above statement in Bordet's own publication, but in discussing the relation between hemagglutination and the sensitization of red blood corpuscles Bordet (1) does state that rabbit red blood corpuscles, although strongly agglutinated by heated chicken serum, are no more susceptible to the slight dissolving effect of normal guinea-pig serum than are normal rabbit corpuscles.

P. Müller (3) verified Bordet's observation, but concluded that the hemolytic action of chicken serum is to be ascribed to an amboceptor-alexin complex, and that the difficulty experienced in reactivating the serum arises from the fact that the amboceptors of chicken serum have complementophile groups which do not react with the haptophore groups of complements from other species. Müller claims to have established this point by showing that inactivated chicken serum can be reactivated with fresh serum from treated chickens and with the serum from the pigeon, a closely related species, but not with serum from other species. Müller's experiments on this point were briefly as follows: Chickens were given intraperitoneal injections (daily for from 1 to 3 days) of bouillon, peptone water or suspensions of aleuronat, and were bled from 12 hours to 3 days after the last injection. The serum from this blood was used as complement, the supposition being that the treatment had increased the complement content of the serum. The serum from untreated chickens was inactivated by heat and used as amboceptor. Müller's protocols show that from 0.05 cc. to 0.07 cc. of the complementing serum plus 0.2 cc. of the heated serum hemolyzed a unit of cells, and that 0.1 cc. of the complementing serum alone also hemolyzed a unit of cells. The protocols on reactivation with pigeon serum show that 0.15 cc. of fresh pigeon serum hemolyzed a unit of rabbit cells with 0.2 cc. of inactivated chicken serum as amboceptor. The hemolytic dose of the pigeon serum alone was about 0.3 cc.

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The object of the present paper is to present new observations on the reactivation of the natural hemolysins of chicken serum for rabbit red cells complemented by the fresh sera of the chicken and the guinea pig after inactivation by heat.

Experimental.

General Technique.—The serum used in the following tests was freshly collected and separated from the clot by centrifugalization. The sensitizing serum, as well as the serum used for complement, was pooled from two or more animals. The red corpuscles were freshly collected in citrate, washed four times in large volumes of 0.85 per cent. salt solution and resuspended in salt solution to a dilution of 1–4 in terms of whole blood. One tenth of a cubic centimeter of this suspension was the unit of cells, while the complements were diluted so that the required dosage was contained in 0.1 cc. The reagents were added at room temperature to small serological tubes and incubated at 37° C. Readings were made after one hour's incubation and are recorded in the table as 4 + complete hemolysis; ±slight hemolysis and 0, no hemolysis.

Hemolytic titer of fresh chicken serum for rabbit red cells.—The hemolytic action of fresh chicken serum for rabbit red cells was determined according to Protocol 1. The first five figures in this protocol following the heading "Serum" represent the indicated amounts of undiluted serum added to the different tubes. The sixth and following tubes received the serum previously diluted 1–16 with salt solution.

PROTOCOL 1.

Fresh chicken serum vs. rabbit corpuscles.

Tubes....	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Serum....	(1–0) 0.6	0.4	0.3	0.2	0.1	(1–16) 0.8	0.6	0.5	0.4	0.3	0.2	0.1	0.05	0
Serum......	(1–0) 0.6	0.4	0.3	0.2	0.1	(1–16) 0.8	0.6	0.5	0.4	0.3	0.2	0.1	0.05	0
Salt solution	0.3	0.5	0.6	0.7	0.8	0.1	0.3	0.4	0.5	0.6	0.7	0.6	0.85	0.9
1–4 corpuscles...	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1 hour reading.......	4+	4+	4+	4+	4+	+	±	±	0	0	0	0	0	0

Many titrations carried out in the same way have consistently shown that 0.1 cc. of fresh chicken serum completely dissolves the dose of rabbit cells used here. In some cases, however, in the higher dilutions more or less marked hemolysis appears after one hour's incubation. In exceptional cases slight hemolysis has appeared after one hour's incubation in doses as small as 0.025 cc., with complete hemolysis in doses of 0.05 cc. Determinations made in the same way for cells of the guinea pig, ox, sheep and pigeon have shown that fresh chicken serum is hemolytic for these cells. Under the conditions of the experiment, however, the dissolving effect of the fresh serum is most energetic toward rabbit cells and least for those of the pigeon.

The reactivation of chicken serum, ordinary method.—Hemolytic tests were made with heated chicken serum and the red cells of the rabbit, in which the fresh serum of the guinea pig and chicken were used as complements in doses of 0.025 cc. Fresh chicken serum was heated to 56° C. for 30 minutes. The heated serum was added to tubes as in Protocol 1, with 0.1 cc. less of salt solution which was replaced with the same volume of complement serum diluted to give the required dosage. Complement and cells were added as in an ordinary hemolytic titration. The tubes were thoroughly shaken and then incubated. No hemolysis was evident at the end of one hour. Tests were also made in which the heated serum, salt solution and cells were incubated one hour before the complement was added. The tubes were then thoroughly shaken and incubated for one hour longer. There was no hemolysis in this case.

So far, it is seen that fresh chicken serum actively hemolyzes foreign red blood corpuscles, particularly those of the rabbit and that the inactivated serum is not reactivated with fresh sera in ordinary hemolytic tests.

The reactivation of chicken serum, new method.—By a modification of the original technique, chicken serum inactivated by heat at 56° C. for 30 minutes was reactivated for rabbit corpuscles with non-hemolytic doses of fresh chicken serum to practically its original hemolytic titer. The test was made by adding the different amounts of heated chicken serum to tubes as indicated under the tube numbers in the table. Salt solution was then added to a volume of 0.8 cc. 0.02 cc. (a sub-hemolytic dose) of fresh chicken serum (complement) was then added. The tubes were incubated for one hour. Rabbit red cells were then added and the incubation was continued for another hour. Hemolysis appeared as recorded under Set 1, Table I. A control set was run in which the cells, heated serum and saline were mixed and incubated for one hour. Two hundredths cc. of fresh chicken serum (complement) was then added and the tubes reincubated for one hour. No hemolysis occurred, as shown in Set 2. The hemolytic titer of the fresh serum which was used as complement was 0.1 cc. (Set 3, Table I). Five hundredths cc. caused only a trace of hemolysis. The amount used as complement (0.02 cc.) was of itself entirely non-

TABLE I.

Reactivation of chicken serum with chicken serum.

Tubes . . .	1	2	3	4	5	6	7	8	9	10	11	12	13
Serum. . . .	(1–0 0.6	0.4	0.3	0.2	0.1	(1–16) 0.8	0.6	0.5	0.4	0.3	0.2	0.1	0.5
Set 1*. . . .	4	4	4	4	4	3	+	±	0	0	0	0	0
Set 2.	0	0	0	0	0	0	0	0	0	0	0	0	0
Set 3.	4	4	4	4	4	±	0	0	0	0	0	0	0
Set 4.	4	4	4	4	4	4	2	±	0	0	0	0	0

* Set 1 shows the titer of fresh chicken serum; Set 2, inactivated chicken serum and fresh chicken serum as complement, ordinary method; Set 3, inactivated chicken serum and fresh chicken serum as complement, new method; Set 4 is the same as Set 1 with the exception that each tube contains 0.02 cc. more of fresh chicken serum than is indicated.

hemolytic. To test further the intrinsic hemolytic power of the complement serum, 0.02 cc. (dose of complement) was added to each of a series of tubes in which the titer of the fresh serum was being determined. This addition only slightly raised the end titer (compare Set 4 with Set 1, Table I). These controls show that reactivation was actually effected.

The results given in Table I were obtained with serum inactivated at 56° C. fifty-three degrees C. is the lowest temperature at which the serum can be inactivated within 30 minutes. It was found that reactivation can be effected by the method just described after the serum has been inactivated at temperatures between 53° C. and 58° C. Serum heated at higher temperatures was not reactivated.

The reactivation of chicken serum with guinea pig serum, new method.—The technique followed here was the same as that used in the experiment just described in which the chicken serum was reactivated with sub-hemolytic doses of the fresh serum of the same species. The results are recorded in Table II. Five hundredths cc. of fresh guinea pig serum was used as the dose of complement. It took four times this quantity of the serum to hemolyze completely a unit of rabbit corpuscles, twice the amount giving only partial hemolysis, and in controls containing one unit of the complement serum no hemolysis occurred. Here, as with the fresh chicken serum as complement, no hemolysis occurred when the three ingredients—cells, heated serum and complement—were put in the tubes at the same time or when the cells and heated serum were incubated before the complement was added. Reactivation was effected, however, when the heated serum and complement were mixed and incubated one hour before the cells were added. In this instance the chicken serum could not be reactivated if heated above 55° C. It has already been pointed out that 53° C. for 30 minutes completely inactivates the serum (see Table II).

TABLE II.

Reactivation of chicken serum with guinea pig serum.

Tubes....	1	2	3	4	5	6	7	8	9	10	11	12	13
Serum....	(1–0) 0.6	0.4	0.3	0.2	0.1	(1–16) 0.8	0.6	0.5	0.4	0.3	0.2	0.1	0.05
53° C.....	+	2+	4+	3+	±	0	0	0	0	0	0	0	0
54° C.....	4+	4+	4+	3+	0	0	0	0	0	0	0	0	0
55° C.....	4+	2+	+	0	0	0	0	0	0	0	0	0	0

SUMMARY.

It has been shown that the natural hemolysins of chicken serum for rabbit corpuscles can be reactivated both with fresh chicken serum and guinea pig serum after heat inactivation. The reactivation is effected by mixing and incubating the inactive serum and complement before the corpuscles are added.

LITERATURE.

1. Bordet, J.
    1899. Agglutination et dissolution des globules rouges. Ann. de l'Inst. Pasteur, XIII, 273.
2. Metchnikoff, E.
    1907. Immunity in infective diseases. Cambridge Press.
3. Müller, P.
    1901. Über Anti-hämolysine. Centralbl. f. Bakt. u. Parasitenkunde, XXIX, 175.

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THE RELATION OF CERTAIN CARRIER CONDITIONS TO THE VIRULENCE OF HEMOLYTIC STREPTOCOCCI.*

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It has been demonstrated by a number of observers that the hemolytic streptococcus is a common inhabitant of the tonsils, especially of the crypts. Smillie (1917) found a large percentage of healthy carriers in a boys' school during an epidemic of sore throat. During the past few years numerous instances of a high incidence of healthy carriage of this organism in army camps have been reported. Nichols and Bryan (1918) report observations on the treatment of chronic carriers. They state that inasmuch as the hemolytic streptococcus appears to have a special predilection for the crypts of the tonsils rather than the surfaces of these and neighboring structures, it is especially difficult to treat carriers of this organism successfully. Their conclusion is that extirpation is the only satisfactory method. Davis (1920) states that the crypts are an almost constant source of hemolytic streptococci.

Studies of extirpated tonsils thus have added to our knowledge of carrier conditions. Since the crypts, in a large number of instances, contain hemolytic streptococci among their apparently normal flora, cultures of the tonsil surfaces and the pharynx, unless carefully made, will fail to reveal true carriers of this organism. Also it consequently appears that carriers of this organism are more common than formerly supposed.

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Altho reports of the incidence of carriers show these facts, studies of the relative virulence of the organisms carried and the relation of the acuteness or chronicity of the carrier state to the virulence of the strain harbored have not attracted much attention. It is a commonly observed fact that as the number of healthy carriers of any pathogenic organism increases in a group or community of individuals an epidemic is to be feared. This has been explained not only for the reason that there has been an increased opportunity for infection but that the virulence has been increased by more or less rapid passage from one individual to another.

The results of this report embody experiments dealing with the relative virulence for mice of strains of hemolytic streptococci obtained from different groups of healthy carriers, strains obtained from excised tonsils and strains from acute respiratory infections.

Source of cultures.

Cultures were obtained from the following groups: (1) chronic carriers, i.e., individuals who had yielded positive cultures of hemolytic streptococcus from their tonsils or pharynx at different intervals for a period of at least three months; (2) early carriers, individuals who had harbored the organisms for only a few days or weeks, and without history of prior infection; (3) cases of incipient acute bronchitis, laryngitis or tonsillitis; (4) cultures from tonsils removed several months following an attack of tonsillitis.

Technic.

In obtaining the first three groups of cultures, the tonsils and pharynx were swabbed and the material streaked on blood agar plates, in the usual manner. Colonies were picked after 18–24 hours incubation and transferred to blood agar slants. In obtaining cultures from the extirpated tonsils cultures were made immediately after removal, both of the surfaces and of the crypts. To obtain the latter the tonsil was cut in half with sterile instruments and material obtained from several crypts. Both tonsils from each case were cultured in this way. It was usually found that the results were the same; occasionally, however, one tonsil yielded hemolytic streptococcus while the other would be negative.

For the determination of the virulence of a given strain the first blood agar slant culture, obtained from the streaked plate, was used.

One fifth of an 18–24-hour growth of such a slant, suspended in salt solution, was injected intraperitoneally into a mouse. Using an amount of culture of this size, the strain was not considered virulent unless the mouse died in 24 hours.

The number of positive cultures obtained from the extirpated tonsils, three to six months following the acute inflammation, is interetsing. As is seen from Table I 96 per cent. of the 75 cases gave positive cultures, a finding in keeping with previous results (3). Furthermore, in over 93 per cent., in all but five of the seventy-five, hemolytic streptococci were present in large numbers, predominating over other organisms present. In two of the negative cases a hemolytic staphylococcus was present as the predominating organism.

TABLE I.

Hemolytic streptococci from tonsils removed three to six months following acute attack.

		Per Cent.
Exterior surface predominant	3	4.0
Crypts predominant	31	41.3
Approximately equal numbers—crypts and surface	36	48.0
Present in small numbers (a few colonies only)	2	2.6
Negative	3	4.0
Total	75	

Virulence tests.

The following table shows the results of the virulence tests with the different strains obtained.

TABLE II.

Virulence tests.

	Virulent.		Non-virulent.		Total Strains.
	No.	Per cent.	No.	Per cent.	
Early carrier cultures	3	12.5	21	87.5	24
Chronic carrier cultures	4	10.0	37	90.0	41
Extirpated tonsils (3–6 months after acute attack	7	14.5	41	85.5	48
Acute respiratory infections (tonsillitis, laryngitis, bronchitis)	19	86.4	3	13.6	22

Discussion.

The difference in virulence for mice between healthy carrier cultures and strains obtained during an acute infection with hemolytic streptococci, as shown in Table II, is marked. While no conclusions concerning virulence for one species can be drawn from evidence of virulence for another species of animal, yet the parallelism between these groups is sufficiently striking to be worthy of consideration. In view of the size of the inoculation it is interesting to find that only a small minority of the healthy carriers harbored strains that were at all virulent for mice, while in the group of cultures obtained from actual infections, the converse was true.

No differences in the virulence for mice of the strains obtained from early and from chronic carriers were observed. The majority of the early carriers in this particular group were associated with the carriers of longer duration and doubtless harbored much the same strains. Furthermore this group contains only individuals who gave no history of clinical infection. However, a comparison of the two groups seems to point to the fact that the duration of a strain in a healthy normal throat has little to do with its virulence as evidenced by mouse inoculation. This seems to be true for healthy throats.

A comparison of the group of strains obtained from acute respiratory infections with those from removed tonsils shows a general difference in the comparative virulence of each group for mice. The majority of the organisms isolated from the inflamed throats and tonsils, i.e., at the time when there is actual infection, were virulent for mice, usually in much smaller doses than the amount, one fifth of a culture, used in the tests. Yet after the infection has been overcome and the carrier state ensues, a marked decrease in the virulence of the strain apparently takes place. It would seem that during the acute infection, while the host is combatting the organisms, the latter, at least those successful in resisting for a time the defensive mechanism exerted by the host, develop an increased invasive power. Later, when the infection has been overcome, the organisms are living on the surface of the normal mucus membranes in a state of symbiosis, doing no harm, because, in the present state of the host's resistance, actual invasion is impossible. Under these conditions the invasive power—the virulence—of the organisms diminishes.

A further point of interest is brought out by Table I. This table seems to show that a high percentage of cases of tonsillitis become chronic carriers of hemolytic streptococci. Removal of the tonsils seems to be the only way to treat such cases, since the organisms occur deep in the crypts and usually in large numbers. While these strains apparently decrease rapidly in virulence for the individual who harbors them and for mice, it is impossible to assume that these carriers are, therefore, harmless to others.

The decrease in virulence following an attack of tonsillitis may be a partial explanation of the small number of generalized infections following removal of the tonsils from three to six months after the acute attack, as compared with the higher incidence when they are removed soon after the actual infection. The organisms are still present but not only has the individual developed increased resistance; the bacteria, as well, have diminished in invasive power.

Summary.

A low degree of virulence of hemolytic streptocicci for mice is found to occur in cultures taken from healthy carriers, as compared with those from cases of actual infection.

No differences in virulence have been observed between strains from early and from late carrier states. The duration of a strain in a healthy throat seems to have little to do with its virulence.

A rather rapid decrease in virulence seems to occur after the acute infection has ended. Chronic carriers of hemolytic streptococci as a result of an attack of tonsillitis show a very small percentage of virulent strains.

A large percentage of cases of acute tonsillitis become chronic carriers of hemolytic streptococci, as evidenced by cultures of tonsils removed three to six months following the acute attack.

BIBLIOGRAPHY.

1. Davis, D. J.
1920. The tonsil in relation to infectious processes. Journ. A. M. A., LXXIV, 317.
2. Nichols, H. J. and Bryan, J. H.
1918. The tonsils as foci of infection in Streptococcus hemolyticus carriers. Journ. A. M. A., LXXI, 1813.
3. Smillie, W. G.
1917. Studies of the beta hemolytic streptococcus. Journ. Inf. Dis., XX, 45.

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A TOXIC SUBSTANCE OBTAINED BY GROWING HEMOLYTIC STREPTOCOCCI IN A SPECIAL MEDIUM.*

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Invasion by the hemolytic streptococcus is responsible for a number of different diseased conditions. Thus, we have "septic sore throat," tonsillitis, bronchitis and broncho-pneumonia—to name but a few of the more common diseases in which the hemolytic streptococcus plays a prominent part. In addition, hemolytic streptococci are responsible, as secondary invaders, for grave complications in other diseases, of which measles may be taken as an example. The severity of the systemic reaction is a striking characteristic of all these infections and, where a fatal termination results, it is often difficult of explanation by the extent of the pathologic process or the apparent degree of the infection.

In addition to these clinical considerations, there are other points which suggest that at least a part of the pathogenic power of hemolytic streptococci is to be ascribed to specific intoxication. Thus, when mice are injected intraperitoneally with several times the lethal dose of a virulent strain, death usually takes place within a few hours. Whether death occurs in two hours or is delayed for twenty-four hours or longer, autopsy reveals little to explain the mechanism of the pathogenic action. The peritoneum contains little or no exudate. Organisms can be recovered from the heart's blood, but smears and cultures frequently reveal the absence of an overwhelming septicemia. Autopsy findings in man are correspondingly unsatisfactory in revealing the manner in which this organism produces its fatal effects.

The present study was undertaken, therefore, in the hope of determining more definitely the mechanism of the pathogenic action of the hemolytic streptococcus.

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Experimental.

The Organisms.—The source and laboratory numbers of the nine strains of hemolytic streptococci included in this study were as follows: Strains 4 and 64 were isolated from the sputa of cases of broncho-pneumonia, the first in January, 1920 and the second in November, 1918; strains 6 and 16 were recovered from throat cultures of cases of acute tonsillitis in February, 1920; strains 9, 34, 41 and 43 came from throat cultures of influenza patients, January, 1920; strain 170 was recovered from a throat culture of a case of acute bronchitis, October, 1918. All of these strains produced typical beta hemolysis on blood agar plates and their morphology, fermentation reactions and insolubility in bile placed them in the hemolytic streptococcus group. These particular strains were selected on account of their original virulence for mice and because it was found possible to raise their virulence by means of animal passage. The final virulence of all the strains was such that from 0.001 cc. to 0.0001 cc. of an 18-hour broth culture killed white mice within from twenty-four to forty-eight hours.

Experiment I illustrates the fact, already referred to, that streptococcus infections may be fatal with comparatively scant growth of the organisms within the host.

Experiment I.—Mouse 1: This mouse was given, intraperitoneally, 0.001 cc. of an 18-hour blood broth culture of strain 9. Death occurred in thirty hours. There was a slight exudate in the peritoneum. Films from the heart's blood showed only an occasional streptococcus.

Mouse 2: Five tenths cc. of a blood broth culture of strain 9 was injected into the peritoneal cavity. The mouse was dead four hours later. The peritoneum was dry. The mesenteric and visceral peritoneum was moderately congested. Films from the heart's blood showed very few organisms. Cultures made from the peritoneal cavity and heart's blood were positive.

The results obtained in Experiment I corroborate the statement made above, that infections by the hemolytic streptococcus may be fatal in the absence of an extensive multiplication of the organism in the tissues of the host. This was true in both instances here noted, one mouse receiving a 30-hour lethal dose and the other mouse 500 times this amount. It is indicated, therefore, that the organism produces a very active poison on multiplication within the tissues of the host, since only a slight growth was necessary to cause death, and since sterile filtrates from the infecting cultures were devoid of toxic action, 2 cc. not being fatal.

Evidence of the production of toxic substances in infected animals leads one to believe that the hemolytic streptococcus (as well as many other pathogenic bacteria) would do the same in vitro if it were grown under the proper conditions. An attempt to devise a medium in which this organism would produce filtrable toxic substances resulted in the preparation of the following medium which was used in the experiments described below: 1 per cent. peptone, 1 per cent. di-sodium phosphate and 0.5 or 1 per cent. glucose are added to an ordinary meat infusion made with distilled water, and the reaction adjusted to a PH of 8.0 to 8.2. The medium is tubed, about 10 cc. to a tube, and autoclaved at ten pounds' pressure for ten minutes. A fragment of sterile rabbit kidney and 1 cc. of defibrinated sheep or rabbit blood are now put in each tube. The medium is then ready for use and, to obtain the best results, should be inoculated at once, without incubation to determine sterility. Contaminated tubes can be detected and discarded as they appear.

In this medium the hemolytic streptococcus grows readily and abundantly. Cultures grown in it for a period of 18 to 36 hours were highly virulent for mice and killed fully as rapidly as when grown in plain meat infusion broth. Occasionally filtrates of such cultures incubated for this length of time will kill when injected in 1 or 2 cc. amounts, but not with any degree of certainty. After 48 hours' incubation there occurs a very marked increase in the toxicity of the centrifugalized or filtered culture and this increases up to 72 or 96 hours, when it begins to decrease. This time curve of the rise and fall of the toxicity of the cultures in this broth has been characteristic of the strains studied. Usually the maximum toxicity is obtained in about 72 hours, when from 0.1 to 0.2 cc. of filtrates from cultures kill white mice within 24 hours.

PROPERTIES OF THE TOXIC SUBSTANCE.

Effect of Filtration.—The passage of cultures through Mandler filters does not destroy the toxic action of the filtrate. While the supernatant fluids of cultures centrifugalized at high speed still contain a few organisms the number present has not been found to interfere seriously with the study of the toxic substance. This substance apparently lacks the characteristic aggressive action of most true toxins known at present and consequently the presence of a few streptococci in the fluid injected does not lead to a fulminating infection. This point will be referred to later in detail. The Mandler filtrates were about one half as toxic as the supernatant fluid. This indicates that a portion of the toxic substance is held back or destroyed by filtration.

Relation of Acidity to Toxicity.—It is well known that streptococcus cultures produce large amounts of acid, and, while the initial reaction of the medium and the buffer action of the phosphate tend to control this acidity, most cultures in three days' time reach a PH of 6.5 to 6.0, and occasionally a higher degree of acidity develops. It was possible, therefore, that the toxic action might be due to the acidity, but neutralization of the fluid with sodium hydroxide does not alter the toxicity.

Specificity.—It was also possible that other organisms grown in this medium, or the medium alone, might display the same toxic effect. However, filtrates of the sterile medium incubated three days, and filtrates of cultures of non-hemolytic streptococci, pneumococci, staphylococci and typhoid bacilli grown in the medium showed no toxic action when injected, even in large amounts, into mice. This is in marked contrast to cultures of hemolytic streptococcus strains, where 0.1 cc. of the filtrate is often fatal to mice in less than twenty-four hours.

Pathogenicity.—As mentioned above, this product is toxic for mice when injected intraperitoneally. There appears to be a definite relation between the amount injected and the time of death. Following the injection of amounts as large as 1 to 2 cc. death sometimes occurs in as short a time as one hour. This is the shortest interval that has been observed with intraperitoneal injections. Following acutely fatal doses, death is apparently due to respiratory failure, the heart having been observed to beat for several minutes after respiration has ceased. When smaller amounts are injected and death is delayed the symptoms are more prolonged. Prostration occurs and the respirations become more and more difficult and labored until the time of death. Autopsy shows a remarkable absence of any gross pathological condition.

The intravenous injection of mice produces almost instantaneous death, the animal often being dead before it can be released following the injection. Even such small amounts as 0.01 cc. have sometimes caused death. If the injection is not immediately fatal the incubation period is variable. The intraperitoneal method gives more satisfactory results, the incubation period and the final results being more uniform.

A small number of rabbits and guinea pigs were injected, but the effect of the toxic substance on these animals is irregular, some being apparently very susceptible and others relatively resistant. The intravenous injection into rabbits of 1 cc. per kilogram of body weight has caused death. The incubation period is usually short, as is the case with mice injected intravenously. The incubation period has been known to vary, however, from five minutes to eighteen hours, and many rabbits do not succumb to the injection. Death is preceded by convulsions and extreme respiratory distress.

Thermolability.—The toxic action of the filtrates is impaired by heating to 55° C. for 30 minutes and is completely destroyed by heating to 62° C. for 30 minutes.

Stability.—The filtrates rapidly lose their toxic properties even when kept sealed and in a cold dark place. Some specimens deteriorate as much as 50 per cent. in a few days' time in the ice box.

Relation of Virulence to Toxicity.—Roughly, the production of the filtrable toxic substance corresponds to the virulence of the strain of hemolytic streptococcus used. The increase of virulence of a strain by animal passage usually is accompanied by an increase in the production of the toxic product, although one strain (64) which was relatively avirulent for mice, produced a potent poison in vitro.

IMMUNIZATION EXPERIMENTS.

Immunization of Rabbits.—The injection into rabbits of sterile filtrates of cultures containing this toxic substance causes the production of neutralizing and protective substances in their serum. The rabbits respond whether injected intravenously or subcutaneously. The following protocols are illustrative of the immunization process.

PROTOCOL 1.

Immunization of rabbits with the filtrates.

Date.	Rabbit 1, strain 9, intravenous.	Rabbit 2, strain 41, intravenous.	Rabbit 3, strain 9, subcutaneous.	Rabbit 4, strain 41, subcutaneous.
4–27–20........	0.25 cc.	0.25 cc.	0.5 cc.	0.5 cc.
4–28–20........	0.25 cc.	0.25 cc.		
4–29–20........	0.5 cc.	0.5 cc.		
4–30–20........			0.5 cc.	0.5 cc.
5– 2–20........	0.5 cc.	0.5 cc.		
5– 3–20........	0.5 cc.	0.5 cc.	1.0 cc.	1.0 cc.
5– 4–20........	1.0 cc.	1.0 cc.		
5– 7–20........	0.5 cc.	0.5 cc.	1.0 cc.	1.0 cc.
5– 8–20........	1.0 cc.	1.0 cc.		
5– 9–20........	1.0 cc.	1.0 cc.		
5–10–20........			2.0 cc.	2.0 cc.
5–17–20........	Bled	Bled	Bled	Bled

The sera obtained from the rabbits of Protocol 1 were tested with respect to their neutralization and protection properties, both against the toxic filtrates and infection with the cultures. Protocols 2, 3, 4 and 5 illustrates the type of experiment used.

PROTOCOL 2.

Neutralization of the poison (Strain 41) in vitro.

Mouse.	Filtrate, strain 41.	Immune serum, rabbit 2.	Normal rabbit serum.	Results.
1.......	0.1 cc.	0	0	Dead in 26 hrs.
2.......	0.1 cc.	0	1.0 cc.	Dead in 28 hrs.
3.......	0.5 cc.	0.5 cc.	0	Survived.
4.......	0.5 cc.	0.25 cc.	0	Survived.

In Protocol 2 the serum and filtrate were mixed and kept at 37° C. for one hour before injection. The mixtures were injected into the peritoneal cavity. It is seen that 0.25 cc. of serum neutralized five lethal doses and that the same amount, or even twice the quantity of normal rabbit serum, had no neutralizing effect upon the toxic filtrate.

Protocol 3 illustrates the protective action of the serum of the immunized rabbits when the serum and the filtrates are separately injected. The serum was injected 8 hours prior to the filtrate.

PROTOCOL 3.

Neutralization of the filtrate in vivo.

Mouse.	Filtrate, strain 9.	Immune serum, rabbit 3.	Normal rabbit, serum.	Results.
1.......	0.2 cc.	0	0	Dead in 20 hrs.
2.......	2.0 cc.	0	1.0 cc.	Dead in 4 hrs.
3.......	2.0 cc.	1.0 cc.	0	Survived.

Protocol 3 shows that 1 cc. of the immune serum protected against ten lethal doses of the filtrate and that the same amount of normal rabbit serum was without effect. Other similar experiments showed that from 0.2 cc. to 0.05 cc. of the sera of the immunized rabbits protected against a lethal dose of the homologous filtrate.

Proportional Neutralization.—In view of the fact that 3 cc. is about the maximum quantity that can be injected into a mouse at one time and since this amount of filtrate would contain hardly more than twenty lethal doses, the following experiment was devised in order to test the proportional neutralizing action of the immune serum on multiple lethal doses of the toxic substance. Thirty cc. of filtrate 9, representing 200 fatal doses, was mixed with 8 cc. of serum 9, 200 neutralizing doses. This mixture was allowed to stand at room temperature for one hour and then 3 cc. were injected intraperitoneally, into each of six mice. The results are shown in Protocol 4.

PROTOCOL 4.

Neutralization of proportional multiple doses of the filtrate.

Mouse.	Amount of Mixture.	Results.
1	3.0 cc.	Survived.
2	3.0 cc.	Survived.
3	3.0 cc.	Dead in 36 hrs.
4	3.0 cc.	Survived.
5	3.0 cc.	Dead in 48 hrs.
6	3.0 cc.	Survived.

It is seen that four of the six mice survived and that the other two lived more than 24 hours in spite of the fact that the mixture which each mouse received contained more than fifteen lethal doses of filtrate. This seems to show that multiples of the toxic filtrate up to at least 200 are quite effectively neutralized by equal unit proportions of the immune serum.

Protection Against Infection.—The following protocol was devised to determine the effect of the serum on infection with cultures of the different strains of hemolytic streptococci included in the study. In this experiment all injections were intraperitoneal and the serum was given 18 hours prior to the culture.

PROTOCQL 5.

Protection against infection.

Mouse.	Culture, strain 9.	Immune serum, strain 9.	Results.
1	0.0005 cc.	0	Dead in 20 hrs.
2	0.0005 cc.	0	Dead in 18 hrs.
3	0.05 cc.	0.5 cc.	Survived.
4	0.05 cc.	0.5 cc.	Suvrived.

This protocol shows that the serum of rabbits immunized with the toxic filtrate protected mice against at least fifty fatal doses of the whole culture. This seems to point to a definite specific relationship between the toxic substance and the organisms.

The Relation of Protection and Serological Grouping.—By means of agglutination and protection experiments with an antibacterial serum produced with strain 9 it was found that at least two serological groups existed among the strains included in this study. Thus, the strain 9 serum agglutinated and protected against strains 4, 6 and 9, while it neither agglutinated nor protected against strains 41, 64 and 170. It was, therefore, of interest to see if this grouping extended to the toxic substances and their immune sera. For this purpose rabbits were immunized with the filtrates from strains 6, 9 and 41 respectively and their sera used in protection tests with filtrates from cultures 4, 6, 9, 41 and 64. The sera and filtrates were mixed in vitro and kept at room temperature one hour before they were injected. The details of the experiment are given in Protocol 6.

PROTOCOL 6.

Cross protection tests with the toxic filtrates and their respective immune sera.

Mouse.	Filtrates.	Immune serum.	Results.
1......	0.5 cc. strain 9	0.5 cc. strain 9	Survived.
2......	0.5 cc. strain 6	0.5 cc. strain 9	Survived.
3......	0.5 cc. strain 4	0.5 cc. strain 9	Survived.
4......	0.5 cc. strain 6	0.5 cc. strain 6	Survived.
5......	0.5 cc. strain 4	0.5 cc. strain 6	Survived.
6......	0.5 cc. strain 9	0.5 cc. strain 6	Survived.
7......	0.5 cc. strain 41	0.5 cc. strain 41	Survived.
8......	0.5 cc. strain 64	0.5 cc. strain 41	Survived.
9......	0.5 cc. strain 9	0.5 cc. strain 41	Dead in 16 hrs.
10......	0.5 cc. strain 41	0.5 cc. strain 9	Dead in 8 hrs.
11......	0.5 cc. strain 4	0.5 cc. strain 41	Dead in 16 hrs.
12......	0.5 cc. strain 41	0.5 cc. strain 6	Dead in 8 hrs.

The results of Protocol 6 show that the sera of the rabbits immunized with filtrates from the strains (two strains) of one serological group protected against filtrates from all the strains of this group but not against filtrates from two strains of the other group. In like manner the serum from a rabbit immunized with the filtrate from a strain of the other group protected against its own filtrate and the filtrate from another strain of this group and failed to protect against filtrates from the first group. It appears then that filtrates from different serological groups have correspondingly different antigenic properties. This part of the work, however, needs to be extended.

DISCUSSION.

The development of the poisonous substance described above has been found to depend upon the cultivation of the hemolytic streptococcus in a special medium. The use of ordinary media of various kinds leads to negative results. The use of special media for the demonstration in vitro of toxic products of the growth of pathogenic bacteria has been found to be of value in a number of instances. Clark and Felton (1) (1918) have suggested that a filtrable toxic substance is produced by the growth of hemolytic streptococci in blood diluted with Locke's solution. Bull and Pritchett (2) (1917) found that fresh tissue was an important constituent of the medium in demonstrating the production in vitro of the toxin of *B. welchii,* and Robinson and Meader (3) (1920) have found that such a procedure enhances the toxicity of the broth in which *B. diphtheriae* is grown.

At present only conjecture can be made concerning the purpose served by the different constituents of our medium. Certain precautions are, however, necessary. Distilled water should be used in the preparation of the meat infusion, since it has been found that certain specimens of tap water have an unfavorable action on the production of the poison. No sodium chloride is added since this salt seems also to have an unfavorable influence upon the production of the toxic substance. The addition of di-sodium phosphate is apparently necessary, but its influence is not entirely clear. It probably acts as a buffer in limiting the amount of free acid present in the culture fluid, since the toxicity of the medium decreases as the acidity rises. The presence of blood is not absolutely essential to the maximum production of the poisonous product, but a medium containing 10 per cent. of defibrinated sheep or rabbit blood has given more constant results than the plain medium. The use of fresh tissue is important. The tissue must be fresh and the medium used immediately.

In describing this filtrable toxic substance care has been taken to avoid the use of the term "toxin." The word "toxin" has come to be regarded as defining specific substances, generally of bacterial origin, which conform to definite criteria, such as high toxicity, a definite and characteristic relation between the so-called Lo and the L + doses, definite antigenic properties and certain other characteristics of equal definiteness. While the toxic product which has been described for the hemolytic streptococcus possesses certain of the properties of a true toxin, such as the neutralization of large amounts by proportionate amounts of immune serum, and the production of immune sera which protect against infection with the cultures, it has been impossible to demonstrate other characteristics of true toxins. The toxic substance of the hemolytic streptococcus lacks high potency, although it compares favorably in this respect with the toxin of

B. welchii. It causes no local lesion and consequently it has been impossible to determine an Lo dose with any consequent differences between the Lo and the L + doses. Furthermore, it apparently lacks the typical agressin action which is a striking characteristic of all other known toxins. The injection of the toxic substance together with, or preceding, the injection of the streptococci themselves, has no marked influence on the course of the infection, aside from that which can be ascribed to the toxic action of the fluid itself.

The specificity of the toxic filtrates seems to have been established. As antigens they stimulate the formation of antibodies which neutralize their toxic action and also the homologous cultures. Further evidence of specificity is afforded by the fact that immune sera produced with filtrates from one serological group do not neutralize the filtrates from another group.

It is impossible to state definitely at the present time what part, if any, this poisonous product plays in natural infections with hemolytic streptococci, but both clinical and experimental evidence seems to point to the action of some such product as at least a part of the process of natural as well as experimental infections with this organism.

Summary.

A special medium has been described in which a specific toxic substance has been produced during the growth of certain strains of hemolytic streptococci.

This toxic product is filtrable and the filtrates have a definite pathogenic action when injected into mice, rabbits and guinea pigs.

The poison possesses definite antigenic properties and the sera of rabbits immunized with such toxic filtrates gives protection both against infection with the cultures and against intoxication with the filtrates.

BIBLIOGRAPHY.

1. Clark, A. H. and Felton, L. D.
1918. A filtrable toxic product of the hemolytic streptococcus. Jour. Am. Med. Ass., LXXI, 1048.

2. Bull, C. G. and Pritchett, I. W.
1917. Toxin and antitoxin of, and protective inoculation against *Bacillus welchii.* Jour. Exp. Med., XXVI, 119.

3. Robinson, G. H. and Meader, P. D.
1920. The use of tissue in broth in the production of diphtheria toxin. Jour. Infect. Dis., XXVII, 106.

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EXAMPLES OF SELECTIVE ADSORPTION BY B. PYOCYANEUS.[1]

By WILLIAM C. MOORE.[2]

ABSTRACT.

A study of the adsorption of hydrochloric, sulphuric and acetic acids by the bacillus pyocyaneus, alone and in the presence of sodium chloride, showing that selective adsorption occurs which is partly physical and partly chemical in its nature; part of the acid is taken up in a free molecular condition. It is shown that the bacteria suspended in water between electrodes migrate toward the anode, indicating that they carry a negative charge. Donnan's theory of "Membrane Equilibria" is used to explain this negative charge. [J. W. R.]

INTRODUCTION.

It has long been known that many bacterial species are "agglutinated" or flocculated by acids within certain ranges of hydrogen ion concentration, the actual effective value for agglutination apparently differing with the different organisms. Thus it was found by Michaelis[3] and his co-workers[4] that in certain acetic acid sodium acetate mixtures typhoid bacilli were strongly agglutinated while colon bacilli and paratyphoid A and B were unaffected. Michaelis' proposal to use this phenomenon as means of identification of typhoid bacteria, however, has never been widely accepted, since agglutination with specific serum is more readily performed. Previous to the work of Michaelis, it was shown by Buxton and his co-workers[5] that adsorption plays an important, if not the essential part, in the agglutination of bacteria by acids, and by certain artificial dyestuffs. Bordet[6] assumed that in the specific agglutination of a bacterium in the presence of sodium chloride, the second stage of the process consists in the adsorption of salt with the result that the electrical charge on the bacteria is neutralized and the bacteria thus agglutinated. Joos[7] has shown qualitatively that such adsorption takes place. Adsorption phenomena have, therefore, long been recognized as playing an important part in agglutination. If sodium chloride is adsorbed when it is present in specific agglutination, to what extent is the agglutinin itself adsorbed? Again, what is the real nature of the agglutination process and on what does it depend? Believing that some light on the solution of these problems might be obtained by a study of the conditions involved in the agglutination of bacteria by inorganic substances, an investigation of this field has been undertaken. The work presented in the present paper concerns the adsorption of certain acids by B. pyocyaneus, and is given here because of its interest from a physico-chemical standpoint.

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[3] Folia Serologica (1911), 7, 1010-1012.

[4] Zeit. Immunitätsforsch (1912), 12, 310.

EXPERIMENTAL.

The general method of procedure was to make cultures of the organisms on beef extract (or infusion) agar in Kolle flasks, wash off the culture in distilled water, wash the bacilli four or more times with distilled water, re-suspend them in distilled water, and filter through coarse filter paper to remove clumps and particles of culture medium.

A migration experiment with an aqueous suspension of B. pyocyaneus, prepared as above, was now carried out using a U-tube of the Burton[8] type. Under the influence of 110 volts direct current, the bacteria migrated toward the anode, thus indicating that they carried a negative charge.

[5] Zeit. Phys. Chem. (1907), **57**, 47-89; *ibid.*, **60**, 469-506.

[6] Ann. de l'Inst. Pasteur (1899), **13**, 225.

[7] Zeit. für Hygiene (1901), **36**, 422-439.

[8] Burton: "Physical Properties of Colloidal Solutions" (1916), **131**.

In the preliminary experiments, a qualitative study of the action exhibited by B. pyocyaneus toward alizarin red was made. It was found that suspensions of B. pyocyaneus which had been thoroughly washed with water and allowed to stand in contact with the water were decidedly alkaline in reaction. When centrifuged, the bacteria carried down part of the dye, which, however, could be removed by two washings with distilled water. On adding water and alizarin red to this centrifuged mass, the reaction was at first neutral, slowly becoming more alkaline. In the presence of N/500 sulphuric acid, a certain experiment showed the bacteria to be alkaline in reaction, while the supernatant fluid was decidedly acid. This held true when the number of bacteria per cubic centimeter was varied from about 5×10^8 to 40×10^8.

Using a different organism, B. proteus vulgaris, it was found that alizarin red is more difficult to remove from the bacteria after they had been previously treated with acids than before being subjected to such treatment. Even when the acid employed was of such a concentration that the bacteria at first showed an acid reaction, subsequent washing with water gave a bacterial sediment alkaline in reaction.

In the presence of sodium chloride, B. pyocyaneus, while still alkaline to alizarin red, took on a deeper shade of color than when treated with water alone.

After a series of preliminary experiments, accurate quantitative data were now obtained on the adsorption by B. pyocyaneus of (1) sodium chloride, and (2) hydrochloric, sulphuric and acetic acids, both in the presence and the absence of sodium chloride. The experiments on sodium chloride in the presence of acids are not reported here, as the results obtained were irregular and must be subjected to further study. In this work suspensions of the bacteria, prepared as described above, were used. In carrying out an experiment, a definite volume of the acid or the sodium chloride solution was run into a large centrifuge tube, then a definite volume of the bacterial suspension was added. Preliminary experiments having shown that considerable evaporation occurs during an experiment of this kind, the tubes in any one series of experiments were quickly weighed, the contents of each tube thoroughly shaken, and the tubes placed in an incubator at 37.5° C. for an hour. On removal from the incubator any agglutination occurring was noted, and the tubes then set in an ice-box over night. The next day each tube was centrifuged until the supernatant liquid was practically clear, and then weighed. By this means a sufficiently accurate measure of the evaporation of water from the tube was obtained, as the weight difference in grams was assumed to be equal to the number of cubic centimeters of water lost by evaporation. This assumption could, of course, be safely made, as volumes were read only to the nearest hundredth of a cubic centimeter, and all measurements were made at room temperature.

A definite volume of the clear, supernatant fluid was now withdrawn and the acid content determined by titration with sodium hydroxide. With hydrochloric and sulphuric acids, methyl orange was the indicator used; with acetic acid, phenol-sulfone phthalein. To determine chloride ion, tenth-normal silver nitrate was used, with potassium chromate as indicator. When both acid and chloride were to be determined, the acid was first determined and then the chloride in the neutralized solution.

By evaporation of a definite volume of the original washed suspension of bacteria, at 70° C., a residue was obtained, the weight of which gave the amount of bacterial substance present. A count of the bacteria per cubic centimeter in the original suspension was also made with a Levy hemocytometer.

The following protocol will serve as an example of the method of work:

Experiment 25.

A three-day-old culture of B. pyocyaneus was suspended in distilled water, washed four times, re-suspended in water, filtered and used in the following protocol with hydrochloric acid. Bacterial count, 3.77×10^9 bacilli per cc. On evaporation of 10.22 cc. to dryness at 70° C., 1×10^{-3} grams of bacterial substance per cc. was obtained.

After mixing the bacteria with the acid, the tubes were incubated one hour at 37.5° C., then set aside in the ice-box over night. They were centrifuged and the acid in 10.22 cc. of the supernatant liquid titrated.

TABLE I.

Detailed Protocol: Adsorption of Hydrochloric Acid by B. Pyocyaneus.

Tube No.	1	2	3	4	5	5
Bacterial Suspension, c.c.	5.00	5.00	5.00	5.00	5.00	5.00
N/20 HCl. (Factor 0.9388) c.c.	8.00	4.00	2.00	1.00	0.80	0.60
H_2O, c.c.	7.00	11.00	13.00	14.00	14.20	14.40
Final conc., HCl, 0.9388 ×	0.02 N	0.01	0.005	0.0025	0.002	0.0015
Weight on mixing, grams	113.27	104.57	111.85	110.58	114.54	115.28
Weight, centrifuged, grams	112.33	103.42	111.00	109.64	113.80	113.81
Loss of Weight, grams	0.94	1.15	0.85	0.94	0.74	1.47
Final Volume, c.c. ...	19.06	18.85	19.15	19.06	19.26	18.53
Calc. NaOH for No Action, c.c.	70.92	35.46	17.73	Spoiled	7.09	5.32
Observed c.c., NaOH.	69.19	33.10	14.60	....	4.78	0.00
Adsorbed Acid, equivalent to NaOH, c.c.	1.73	2.36	3.13	....	2.31	5.32
Total Acid Adsorbed, × 10^6 moles, c.c....	8.86	12.08	16.03	....	11.83	27.2
Percent Acid Adsorbed	2.44	6.65	17.60	....	32.57	100.0
Acid Adsorbed × 10^6 moles per 10^{10} bact.	4.75	6.47	8.59	....	6.34	14.40
Acid Adsorbed × 10^3 moles per gram dry bact.	1.77	2.41	3.20	....	2.36	5.44

The results obtained are shown in summarized form in the following Tables:

TABLE II.

Adsorption by B. Pyocyaneus of Sodium Chloride.

Original Salt Conc.	29	60	89	119	147	175	238	263	350	Amount Bacteria per cc.
Salt adsor'd (a)	—186.0	0	0	—97.0	0	0	—150	0	—1027.0	4.4 × 10^9
Salt adsor'd (b)	14.5	0	0	—7.5	0	0	—11.5	0	—78.9	1.145 × 10^{-3}

Salt concentration expressed in milli-equivalents per liter. Line (a) shows salt adsorbed in moles × 10^6 per 10^{10} bacteria; line (b) salt adsorbed in moles × 10^3 per gram of bacterial substance. (—) means "Negative adsorption."

In Table II the figures given show that sodium chloride in concentrations from 29 millimoles per liter up to 350 millimoles per liter is not adsorbed by B. pyocyaneus. In fact, there is some negative adsorption, *i. e.,* in the presence of salt, at certain concentrations, water is taken up by the organisms.

TABLE III.

Adsorption by B. Pyocyaneus of Acids from Aqueous Solutions.

Concentrations Used	1.0	1.50	2.0	2.50	5.0	10	20	Amount Bacteria per cc.
H_2SO_4; factor 1.0229 (a)	2.93	2.47	3.03	13.77	5.35	9.60	3.23	*1.35×10^9
H_2SO_4; factor 1.0229 (b)	1.10	0.93	1.14	5.81	2.25	4.05	1.36	*1.6×10^{-3}
HCl; factor 0.9388 (a)	2.96	14.4	6.34		8.59	6.47	4.75	3.77×10^9
		3.78	3.54	3.44	3.62	2.59	0.86	5.29×10^9
HCl; factor 0.9388 (b)	1.79	5.44	2.36		3.20	2.41	1.77	1×10^{-3}
		2.32	2.13	2.07	2.18	1.56	0.52	0.88×10^{-3}
$HC_2H_3O_2$ factor 1.153 (a)	17.60	17.0	21.6	26.2	41.2	15.80	89.6	0.824×10^9
	4.8	3.26	3.49	7.46	6.66	†8.79	9.66	2.64×10^9
$HC_2H_3O_2$ factor 1.153 (b)	2.14	2.06	2.62	3.17	5.0	1.91	10.9	0.68×10^{-3}
	2.88	1.92	2.10	4.49	4.0	5.28	5.80	0.44×10^{-3}

Figures at head of columns express concentrations in milli-equivalents per liter. Lines (a) and (b) have the same significance as in Table II.

* For concentrations 1.0, 1.5 and 2.0 the figures were the same as for the hydrochloric acid—3.77 x 10^9.

† Concentration was 8, and bacterial dosage twice that in other tubes. Volume was 25 cc.

Table III shows that with hydrochloric, sulphuric and acetic acids there is definite, positive adsorption of the acids concerned, at every concentration studied. This adsorption, based on the amount adsorbed per gram of bacterial substance, is of the same order of magnitude for the three acids, and in each case at least one maximum of adsorption is shown. Similarly, in Table IV it is seen that in the presence of sodium chloride adsorption still occurs and maxima of adsorption are still present. However, a comparison of Tables III and IV shows that the general tendency of sodium chloride is to decrease the amount of adsorption of sulphuric acid, and acetic acids, and possibly to increase it with hydrochloric.

TABLE IV.

Adsorption by B. Pyocyaneus of Acids from Sodium Chloride Solution.

Concentrations Used	1.0	1.5	2.0	2.5	5.0	10.0	20.0	Amount Bacteria per cc.
H_2SO_4 (Factor 1.0229) (a)	5.14	5.80	6.08	7.20	6.86	4.24	3.06	2.04×10^9
H_2SO_4 (Factor 1.0229) (b)	1.12	1.26	1.32	1.56	1.49	0.91	0.66	9.4×10^{-4}
HCl (Factor 0.9388) (a)	10.50	12.20	15.00	13.20	15.20	9.70	9.10	2.04×10^9
HCl (Factor 0.9388) (b)	2.28	2.64	3.27	2.87	3.31	2.11	1.95	9.4×10^{-4}
$HC_2H_3O_2$ (Factor 1.153) (a)		. . .	16.60	18.60	5.58	15.60	5.72	0.824×10^9
$HC_2H_3O_2$ (Factor 1.153) (b)		. . .	2.02	2.26	0.67	1.89	0.69	0.68×10^{-3}

Figures at heads of column and (a) and (b) have same significance as in Table III.

Sodium chloride present; with H_2SO_4, 0.147N; with HCl, 0.147N; with $HC_2H_3O_2$, 0.1456N.

In Tables II, III and IV, inclusive, the total volume employed was 20 c.c.

DISCUSSION OF RESULTS.

The three possible types of action when bacteria are mixed with an acid are:

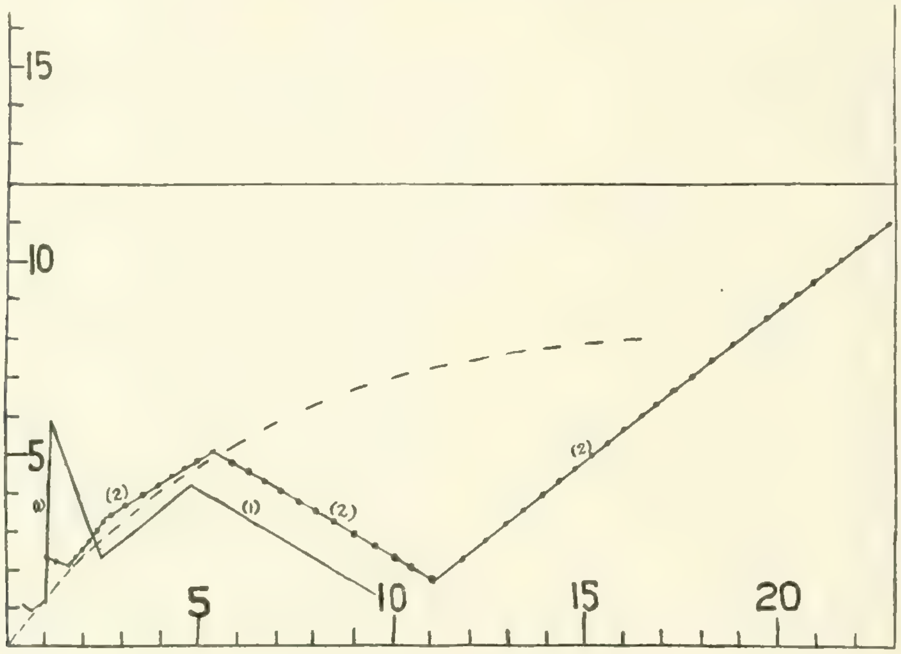
1. Simple adsorption. This may be (a) of the molecular acid, or (b) of the ions of the acid, or (c) of a mixture of molecules and of ions.

2. Chemical combination of the acid, as a molecular species, or of its ions, with the protein of the bacterial substance.

3. Catalytic hydrolysis of the protein. This type of action is probably largely due to the hydrogen ions present. If carried to an extreme it would result in the formation of free amino acids capable of neutralization by sodium hydroxide, thus giving an increase in the titrable acid present.

We shall now discuss the above possibilities in turn.

The modern view of adsorptive processes is that chemical forces hold the adsorbed substance on the surface of the adsorbent.[9] A chemical action of this type would be different from that of type 2, in that the action would be limited to the surface involved. As protoplasm is colloidal in nature, the internal surfaces of the bacterial bodies are probably very great in extent as compared with the external surface. Due, however to the small size of the organisms, any one of the suspensions we have used exhibits a very considerable external surface as the following calculation shows: Park and Williams[10] give the dimensions of B. pyocyaneus as 0.6 μ to 1.0 μ thick and 2 to 6 μ long. Taking the average diameter as 0.8 μ and the average length as 4 μ, the external surface for a single organism, regarding it as a cylinder, is 1.1×10^{-7} sq. cm. In one of the experiments with sulphuric acid, 5 cc. of a suspension containing 1.354×10^{9} bacteria per cc. was used. The number of organisms involved was therefore 5 ×



Legend for Fig. 1.

Abscissa, concentration of acid employed in millimoles per liter. Ordinate, adsorption per gram of bacterial substance in millimoles. Curve 1, for sulphuric acid. Curve 2, for acetic acid. The dotted curve indicates the general type of curve exhibited by the "adsorption isotherm."

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[9] Langmuir: J. Am. Chem. Soc. (1917), 39, 1848-1906.

[10] "Pathogenic Micro-organisms" (1914), 383.

1.354×10^9 or 6.77×10^9. The total superficial area of the bacteria in this experiment is therefore $6.77 \times 10^9 \times 1.1 \times 10^{-7}$ or approximately 750 sq. cm. This is probably only a small part of the total area, internal and external, available for adsorption. Simple adsorption of this type should theoretically follow the course of a curve similar to the well-known "adsorption isotherm." The results obtained in this paper do not follow such a curve, as may be seen by an inspection of Fig. 1, where the dotted curve indicates, roughly, the course of an adsorption isotherm.

Chemical combination due solely to the available hydrogen of the acids employed will not explain the results we have obtained. For example, in one experiment cited in Table III, 5.81×10^{-3} moles, or 11.62×10^{-3} equivalents of sulphuric acid were taken up by 1 gram of bacterial substance. This means that 11.62 equivalents of acid would "neutralize" 1,000 grams of bacterial substance, and hence the equivalent weight of bacterial substance in this instance would be 1,000 divided by 11.62 or 86.06. In order for a protein to exhibit as low an equivalent weight as this, it would have to be completely hydrolyzed into its constituent amino acids. Indeed, of the amino acids making up the proteins,[11] alanin, $C_3H_7NO_2$ is the acid having a molecular weight nearest 86.06, and no protein is known which is built up solely from this substance. Again, in order to accomplish any great degree of hydrolysis of a protein, much more concentrated acids must be used than we have employed in this work.

Combination of the molecular acids employed, however, does not involve any of our ordinary conceptions of valence. Moreover, with acids differing as widely in activity as hydrochloric and acetic, the order of magnitude of the amount of acid adsorbed per gram of bacterial substance is the same. This is strongly indicative of the molecular substances themselves being involved in the action of the acids on the bacterial bodies. It cannot be simple adsorption alone, though this is not precluded. The evidence here presented would seem to indicate that both adsorption and chemical combination take part in the action of acids on B. pyocyaneus.

With respect to the catalytic hydrolysis of the protein substance in the bacteria, such hydrolysis is ordinarily accomplished by boiling with a concentrated acid. The possibility is not excluded, however, of hydrolysis of this type taking place in dilute solutions, at a low rate. It would therefore be the greater the more concentrated the acid solution employed, and, as it results in the splitting of proteins into free amino acids, would give an increase in the titrable acid—an apparent decrease in the amount of acid adsorbed in the higher concentrations of acid.

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[11] Robertson: "Physical Chemistry of the Proteins" (1918), 6.

The alkaline reaction of an aqueous suspension of B. pyocyaneus, and the increase in alkalinity of such a suspension on standing after thorough washing with water, indicates another type of hydrolysis, explicable by Donnan's theory of "Membrane Equilibria.[12] According to this theory, if a constituent of the cell ionizes into a non-diffusible organic radical R^-, and a positive radical M^+, M^+ and OH^- from the water within the cell diffuse out of the cell into the water, leaving H^+ and R^- within the cell. We know, however, that in the case of the proteins HR is a weak acid, so the hydrolysis would in all probability be greater than demanded simply by Donnan's theory. That the OH^- ions are present on the surface of the bacterial cells, may be the cause of the cells in aqueous suspension carrying a negative charge of electricity.

The Tables may now be briefly discussed. The maxima of adsorption shown by sulphuric, hydrochloric and acetic acids in Tables III and IV indicate that in their action upon B. pyocyaneus simple adsorption is not entirely responsible for the results obtained. This, and the calculations above, from the basis of the conclusion that chemical forces help determine the amount of acid adsorbed per gram of bacterial substance.

The exact location of these maxima may not be of significance, but if we express the original concentration of the sulphuric acid employed in molar instead of equivalent quantities, it is seen that one of its maxima coincides with that of hydrochloric and acetic acids. (See also Fig. 1.) This again suggests the participation of molecular substances in the reaction.

No adequate explanation of the effect of salt on the adsorption of the acids has offered itself. It is of course possible that it decreases the degree of dissociation of the hydrochloric acid, causing an increase in the adsorption of the molecular substance, and that its ions react to some extent with the sulphuric acid forming some sodium hydrogen sulphate and lessening the amount of free sulphuric acid available for adsorption; but with a weak acid like acetic such an explanation is not tenable.

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[12] See the interesting discussion in Vol. II, Lewis' "System of Physical Chemistry" (1919), 275.

Its action in increasing the adsorption of water does not at present yield to any simple explanation. In amount, the greatest excess found was where 0.35 molar salt was used, the excess being 78 9 millimoles per gram of bacterial substance. The total salt present was found to be equivalent to 92.18 cc. tenth-normal silver nitrate, the calculated amount being 87.66 cc., an excess of 5.15 percent. As the evaporation was taken into account by weighing, any change in volume due to compression of the water in its adsorption on the bacteria could not be ascertained in this way. This whole question of the action of salt is to be gone into much more thoroughly than we have at present done.

SUMMARY.

In conclusion, this paper shows that:

1. Treatment of B. pyocyaneus with hydrochloric, sulphuric and acetic acids, alone and in the presence of sodium chloride, results in selective adsorption of the acids employed, in that the adsorption found does not follow the adsorption isotherm.

2. The adsorption is partly physical and partly chemical in nature.

3. In the adsorption, part of the acid is taken up in a free molecular condition.

4. Sodium chloride is not readily adsorbed by B. pyocyaneus.

5. An application of Donnan's theory of "Membrane Equilibria" is made to explain the alkalinity of suspensions of B. pyocyaneus in water, and to explain the negative charge on these organisms.

DISCUSSION.

W. C. Moore: I will just take a few minutes to explain why we began this work, and say something as to what it means. If you take an animal like a rabbit or a guinea pig, and give such an animal by means of several injections, either intravenously, interperitoneally or subcutaneously, suspensions of certain species of germs, as typhoid, or any other individual species, you will find that the serum of the blood taken a week or so after the last injection will precipitate out, even in very high dilutions, the specific bacterium that you use for the inoculation of the animal. This phenomenon is known as agglutination. Since the bacteria themselves are so small, they behave really as suspensoid colloids; and the bacterial bodies being composed of protoplasm, which we know is an emulsoid colloid, the problem of determining just how and why the agglutination of bacteria takes place by the specific serum which is developed by using certain definite bacteria is of interest from a colloid standpoint. The thing we are most interested in is to find out why it is that a rabbit made immune to typhoid will give a serum which will not agglutinate other bacteria, and why a rabbit or other animal made immune to certain other bacteria, as for instance pyocyaneus, will not agglutinate typhoid bacteria. The first thing we ran against was this phenomenon known as acid agglutination. I found that a great deal of experimental work had been done by Michaelis[1], but it was not of such a type that you could follow the reaction. We therefore started out with several species of micro-organisms: typhoid, colon, this particular pyocyaneus, and another bacillus known as proteus vulgaris, but found that pyocyaneus was a little more convenient to study than any of the others. In the first place it is easily grown; in the second place, it is easily handled and is not particularly dangerous unless you get it in an open wound; and in the third place, in washing it in distilled water, you can centrifuge the bacteria to the bottom of the vessel very quickly. If you take a culture of this particular bacillus and suspend it in distilled water, you can wash it four or five times in as many hours, almost completely or practically completely free

[1] Folia Serologica (1911), 7, 1010-12

of salt. If you take certain other bacteria, for instance the colon bacillus, it sometimes takes as long as a week to completely wash that organism free of salt, because it settles at a very slow rate. That is how and why we began the work. As far as experimental details are concerned, some points have come up since the paper was in print, which are even more important from the colloid standpoint than the paper itself. In the first place, no reference is made in the actual paper to the agglutination phenomena themselves We purposely left out the order of agglutination in these experiments, because we did not know enough about the results we were getting to commit ourselves. We do not know much more now, but some of the results are of interest. If you will note the table which is given, you will find that the order of adsorption of these acids is sulphuric, acetic and then hydrochloric. Since the paper was printed, we have carried out some experiments with this same bacillus with oxalic acid and find that it comes in between acetic and hydrochloric. When we study bacterial agglutination, we find that flocculation in these experiments follows directly the degree of dissociation of the acid: that is hydrochloric acid flocculates these bacteria most; then sulphuric acid, then oxalic acid and then acetic acid. When we found that out we went on with the colon bacillus, and got some exceedingly interesting results. Instead of following the order here, the colon bacillus followed the order of the degree of dissociation of the acid in the amount of the adsorption. There was most hydrochloric adsorbed; then oxalic, then acetic. However, with the flocculation, the sulphuric flocculated most, then oxalic, then acetic, and finally the hydrochloric. When we arranged the results in that order, it was plain to see what had happened; the colon bacillus apparently had an iso-electric point which is slightly on the alkaline side of neutrality, and in using hydrochloric, sulphuric and oxalic acids, the hydrogen ion concentration is sufficiently great so that the bacteria are peptized. The negative ions of sulphuric and oxalic precipitate these positively charged particles, because these ions are di-valent, and have a precipitating power much greater than you can have in the case of the negative ions of hydrochloric and acetic acid. Acetic, being a weak acid, barely neutralizes the bacterial charge and hence gives more complete flocculation than hydrochloric, which very strongly peptizes the bacteria.

W. D. Bancroft[2]: You will please note that colloid electrochemistry includes pretty nearly everything from wood pulp through electrodes to the inoculation of rabbits. This paper is before you for discussion. I would like to ask whether you imagine that quantitatively the action is proportional to the electrolytic dissociation, or whether it is simply qualitatively?

W. C. Moore: It is closely qualitatively, so far as we know.

W. D. Bancroft: That is what I thought it must be.

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[2] Professor of Physical Chemistry, Cornell University.

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[CONTRIBUTION FROM THE DEPARTMENT OF IMMUNOLOGY, SCHOOL OF HYGIENE AND PUBLIC HEALTH, JOHNS HOPKINS UNIVERSITY.]

OBSERVATIONS ON THE ZINC ELECTRODE.

BY WILLIAM C. MOORE.

Received October 6, 1920.

In a problem recently undertaken in this laboratory, it became necessary to measure small changes in concentration of zinc ion. As both the actual amount of zinc and the concentration changes involved were low (the former varying from the zinc contained in 10 cc. of $0.10\ N$ zinc sulfate solution down to that contained in 10 cc. of $0.0025\ N$ zinc sulfate solution, the latter varying by not more than a few per cent. of these amounts) it was thought that the zinc concentration cell might be used to accomplish the desired end.

A study of the literature, however, showed that all previous investigators had confined their attention either to dilute zinc amalgams of varying composition[1] and constant concentration of electrolyte, or to zinc amalgams or amalgamated zinc, with zinc salt concentrations not less than $0.5\ N$.[2] Moreover, in practically all instances it was found necessary to take some sort of precaution to prevent the access of atmospheric oxygen to the electrolyte, and to the electrode. Thus, Kistiakowsky,[3] who worked with normal zinc solutions, found that constant potentials could be secured by coating the electrode metal with paraffin, placing small pieces of zinc in the electrolyte and sealing the cell with paraffin oil. Richards and Forbes, in their research, protected the amalgams by using an atmosphere of hydrogen.

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[1] Richards and Forbes, *Carnegie Inst. Pub.*, **56** (1906).

[2] Mellenkamp, *Phys. Rev.*, **29**, 329–50 (1909).

[3] Kistiakowsky, *Z. Elektrochem.*, **14**, 113–121 (1908).

In the present work, which deals with the electrode potential of amalgamated zinc rods in solutions of zinc sulfate varying in concentration from 0.5 *N* down to 0.002 *N*, it was found that with cells of the Kistiakowsky type, constant electromotive forces were impossible of attainment. In fact, only after considerable preliminary experimentation was it found that zinc electrodes reproducible at room temperatures to within ± 1 millivolt could be secured, by using well amalgamated zinc rods, and by bubbling hydrogen through the zinc half-cell during the measurements.

Indeed, unless these precautions are taken when electrodes containing 0.1 or 0.01 *N*, zinc sulfate are suddenly diluted to a lower concentration, the electromotive force may occasionally change in the wrong direction. If it is desired to study the electromotive force of such a "dilution cell," it is necessary to use water recently boiled, and at the same time carry out the dilution in such a way that at no time does air come in contact with the water used, after it has been boiled. This can easily be done, by fastening a buret into the stopper of the zinc electrode half-cell, filling with recently boiled water, and passing a stream of hydrogen through this water and through the half-cell previous to and during the dilution.

The measurements recorded below were made with observance of the above described precautions, using a Leeds and Northrup potentiometer, with a Weston cadmium cell as a standard. The zinc of a high degree of purity was kindly furnished by Mr. F. C. Breyer, of the New Jersey Zinc Company, Palmerton, Pa.

In Table I, the column headed "Obs. e. m. f." gives the observed values of electromotive forces for the combination: zinc | zinc sulfate | saturated potassium chloride | 0.1 *N* potassium chloride-calomel | mercury. It was intended to repeat the measurements when a constant temperature bath had been secured, but a change of employment of the writer has prevented this. Mellenkamp, in the article cited above, has shown that with an amalgam containing about 1% zinc, the temperature coefficient of the electrode potential of zinc is small, while the present work shows that changes of several degrees in temperature probably have only a slight effect. Accordingly, the measurements as given were made at "room temperature," which is given in each case. Each figure is a mean of at least 2 and generally several very closely agreeing measurements, made on half-cells assembled just before use.

The column headed "*E* obs." contains the electrode potentials of the zinc electrode at the concentrations given, on the basis of 0.3385 volt for the 0.1 N calomel electrode, referred to the hydrogen electrode as a standard.[1]

TABLE I.

Electrode Potentials of Zinc Electrode.

Electrode No.	Conc. N	Temp. ° C.	Ob. e. m. f.	E obs.
19	0.5	21.3	1.1221	—0.7836
19	0.5	22.0	1.1220	—0.7835
20	0.5	22.0	1.1242	—0.7857
19	0.1	21.8	1.1387	—0.8002
19	0.1	22.9	1.1378	—0.7993
20	0.1	22.0	1.1386	—0.8001
20	0.1	22.6	1.1397	—0.8012
21	0.01	25.5	1.1614	—0.8229
22	0.01	20.8	1.1630	—0.8245
21	0.002	26.4	1.1793	—0.8408
22	0.002	20.1	1.1785	—0.8400

If we assume that the mean value for the 0.1 N zinc sulfate electrode is correct, and apply the Nernst electromotive formula $E = 0.0292 (\log C_1 - \log C_2)$ for concentration cells containing divalent ions, to the 0.5, 0.01 and 0.002 N cells, we get the values shown in the column marked "E calc." in Table II. There is considerable discrepancy between the observed and calculated values. If, however, we take into account the electrolytic dissociation of zinc sulfate, as shown in Table II, Col. 4,[2] we can calculate the electrode potential of the zinc electrode, not for the *total* salt but for the zinc ion at the concentration given. Doing this, we get the values shown in Col. 5, Table II. Applying the Nernst electromotive formula to these electromotive forces, and still using the 0.1 N zinc electrode as standard, we get the values shown in Table II, Col. 6. As is seen, the agreement is good.

TABLE II.

Conc. N.	Mean obs. E.	E calc.	α.	Obs. E_{100}.	Calc. E_{100}.
0.5	—0.784	—0.780	30	—0.769	—0.770
0.1	—0.800	—0.800	45	—0.790	—0.790
0.01	—0.824	—0.829	70	—0.820	—0.819
0.002	—0.840	—0.850	85	—0.838	—0.839

Summary and Conclusions.

1. Observations of previous workers are confirmed in that it has been found necessary to exclude oxygen as completely as possible in setting up zinc electrodes.

2. In the concentration ranges 0.5 N to 0.002 N, the method of coating the metal with paraffin, covering the electrolyte with paraffin oil and placing scrap zinc in the electrode cells, as advocated by Kistiakowsky, is insufficient to accomplish this end.

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[1] Clark and Lubs, *J. Biol. Chem.*, **25**, 479–509 (1916).

[2] Jones and West, *Am. Chem. J.*, **34**, 357 (1905).

3. Electrodes reproducible to within ± 1 millivolt can be secured by making the dilute solutions with water recently boiled and cooled in hydrogen, the dilution taking place in an atmosphere of hydrogen, with the measurements being made while hydrogen is bubbling through the electrode vessel.

4. Using the precautions just described, the electrode potential of zinc in 0.5, 0.1, 0.01 and 0.002 N concentration of zinc sulfate have been measured at room temperature. Calculated values based on the electrolytic dissociation of the zinc salt and these measurements are in good agreement with each other.

5. The Nernst electromotive formula is applicable within the range quoted.

6. In order to measure very small changes in zinc-ion concentration, the zinc electrode even with the precautions described, is not satisfactory. Possibly dilute zinc amalgam electrodes, with extreme precautions for the avoidance of oxygen, would serve the purpose.

Measurements of *Trypanosoma Diemyctyli* from Different Hosts and Their Relation to Specific Identification, Heredity and Environment

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MEASUREMENTS OF *TRYPANOSOMA DIEMYCTYLI* FROM DIFFERENT HOSTS AND THEIR RELATION TO SPECIFIC IDENTIFICATION, HEREDITY AND ENVIRONMENT

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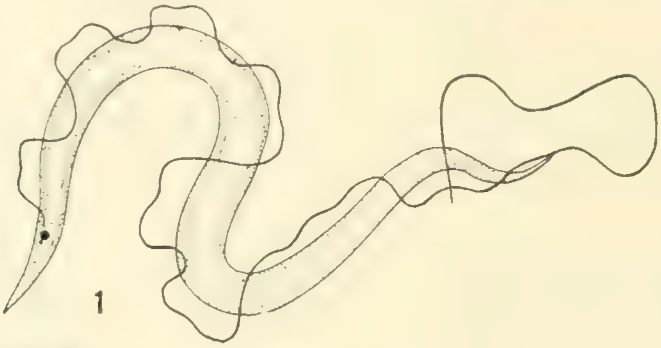
INTRODUCTION

The problems involved in studies of strains of trypanosomes are both scientific and practical. Considerable time and effort have been devoted to attempts to find size differences between human trypanosomes of supposedly different species. Investigators have endeavored also by means of measurements to establish the identity of human trypanosomes with those of certain lower animals and thus to locate animal reservoirs. Furthermore, the data resulting from these researches throw some light upon the possibility of the existence of heritably diverse strains within a species, and upon the effects of the environment, *i. e.* the blood stream, of different species of hosts and of different hosts of the same species on a single strain. It is this last problem that is considered in the following pages.

Trypanosoma diemyctyli (Figs. 1 and 2) was found by Tobey in 1906 to be present in all of a number of newts purchased in an animal store in Boston. During the spring months of 1919 the writer examined the blood of six different species of salamanders. In none of these were any organisms found except in *Diemyctylus viridescens*. All of seventy-eight aquatic specimens of these were infected and two out of seven land specimens. The negative animals were 46 specimens of *Necturus maculosus*, six of *Plethodon glutinosus*, five of *P. cinereus*, 11 of *Desmognathus fusca*, and six of *Spelerpes bilineatus*.

The difference between the aquatic and land forms of *Diemyctylus viridescens* as regards infection with trypanosomes presents an interesting problem. The life cycle of these amphibia includes a year in the water, then a second year on land, and finally a return to the water for mating in the third year. Evidently while on land the infection with the trypanosomes is much decreased. The small numbers of organisms found in these terrestrial specimens may be due to the absence on land of the transmitting agent, which is unknown. The universal abundance of the trypanosomes in the aquatic specimens may be due to their continued inoculation with young stages that have developed in the intermediate host; and the lesser numbers in the land forms, to a gradual dying out of older trypanosomes. It is of interest in this connection to note that of 34 "land" frogs examined during the spring and summer of 1919 only two were infected with trypanosomes whereas of 41 "water" frogs 28 were infected.



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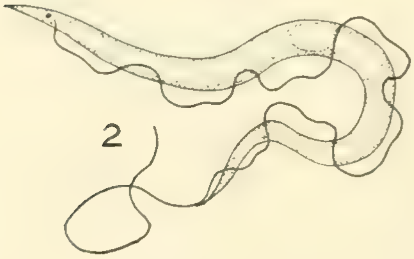

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Explanation of Figures

Fig. 1.—Typical specimen of *Trypanosoma diemyctyli* from newt 19. × 1600.
Fig. 2.—Typical specimen of *Trypanosoma diemyctyli* from newt 15. × 1600.

METHODS OF MEASURING TRYPANOSOMES

In any investigation involving measurements the accuracy of the results depends primarily on the accuracy of the measurements. Trypanosomes are difficult to measure precisely, since their bodies are almost always thrown into curves when fixed. This is especially true of long slender forms. Several methods of obtaining accurate measurements have been employed by investigators.

The method adopted by Bruce, Hamerton and Bateman in 1909 was to draw an outline of each specimen with a camera lucida at a magnification of 2000 diameters "and then to measure along the middle line of the body by means of a pair of fine compasses, the points of which are separated 2 mm. Each step the compass takes is therefore equal to 1 micron."

A modification of this method was employed by Stephens and Fantham (1912). They projected the trypanosomes on a screen with a microprojection apparatus and then traced their outlines with a sharp pencil. A magnification of 2500 diameters was adopted. The drawings were then measured by placing over them semitransparent tracing paper on which a straight line was drawn in ink. One end of the ink line was placed on one end of the drawing and rotated whenever the axis of the trypanosome curved. When the end of the drawing was reached the distance was measured with a millimeter scale.

The method used by the writer seems more desirable than those described above. The trypanosomes were projected with a camera lucida upon a drawing card at a magnification of 1600 diameters. The anterior and posterior ends and kinetonucleus were then indicated with a dot; the width of the body at the nucleus was recorded by two short parallel lines; and the nucleus was drawn. A single line was then drawn down the center of the body from the posterior end to the anterior end. With a chartometer or "map measurer" the distances were easily and accurately obtained.

INVESTIGATIONS INVOLVING TRYPANOSOME MEASUREMENTS

Bruce and his colleagues have measured thousands of trypanosomes of various species in their endeavor to distinguish by size characteristics between the species pathogenic in man and those that occur in the lower animals. The organisms measured were derived from various strains and were taken from a number of species of both wild and laboratory animals. Bruce finally decided that this method of specific identification could not be depended on.

Data regarding the effects of different hosts on the size of the specimens have been provided by various investigators. Thus Laveran and Mesnil (1912) noted a difference between the length of specimens of *T. brucei* grown in the horse and those grown in rodents. Duke (1912) has suggested that strains of numerous varieties exist among trypanosomes of any species, and that alterations in the morphology of a strain may follow continued passage through laboratory animals.

It seems probable from the work of Miss Robertson (1912) on *T. gambiense* that one difficulty in biometric studies of such trypanosomes, is the presence of an endogenous cycle in mammals which results in changes in the types at intervals that cannot be determined by the date of infection. Even if diversities in size were noted in specimens from different hosts the results would not be conclusive. It seems, therefore, that measurements of these polymorphic species are of doubtful value and that better results may be expected when monomorphic forms are studied.

Pearson (1914) has made a biometrical study of many of the measurements published by other investigators and concludes that actual statistical analysis does not in any way confirm the bulk of the conclusions reached by Sir David Bruce and his collaborators. He points out the fact that the data available do not provide material for an analysis of the relative influence of the various environmental factors and hence one cannot determine whether divergences indicate different strains or merely modifications due to different environments.

Most of the quantitative studies of trypanosomes deal with attempts to secure data that will provide means of specific diagnosis. Those data that might furnish evidence of diversities due to the character of the host in which the specimens were grown are of doubtful value because most of the species studied have been dimorphic or polymorphic and hence have exhibited great variations even from a single host. Furthermore, many of the strains measured had received dissimilar treatment; some were taken directly from wild animals, whereas others had been passed through series of laboratory animals of different species during periods of varying length. This treatment may have had an influence on the morphological characteristics of the strains used. The available measurements of monomorphic trypanosomes do not exhibit variations that make possible any definite conclusion as regards diversities when grown in different hosts.

A review of the literature, especially the paper by Pearson, emphasizes the importance of more careful studies of the relations between trypanosomes and their environment represented by different species of hosts and by different individuals of one host species. It is possible to isolate single trypanosomes and to obtain in one host animal a supply of specimens that can be compared with the descendants of other single specimens in host animals of the same or other species. Work of this character is now in progress in this laboratory.

TRYPANOSOMA DIEMYCTYLI FROM DIFFERENT HOSTS

Measurements were made of 100 specimens of *T. diemyctyli* that were taken at random, ten from each of ten individuals of *Diemyctylus viridescens*. Some of these measurements are presented in Tables 1 and 2. The preparations were all made on the same day and stained with Wright's stain. No selection was made either of the newts from which the trypanosomes were obtained or of the trypanosomes on the slides. The first ten trypanosomes that were found in the preparation from each newt were drawn at a magnification of 1,600 diameters and then measured with a map measurer.

Table 1 includes the variations in the distances from the anterior end of the body to the center of the nucleus, from the center of the nucleus to the kinetonucleus,* and from the kinetonucleus to the posterior end. The width of the body at the point where the nucleus is situated is also given as well as average distances. The data are arranged in a series with those of the largest set of ten trypanosomes at the top of the table and those of the smallest set of ten at the bottom. The final averages differ somewhat from those given by Tobey. Tobey's specimens were from 45 to 50μ in length with a flagellum 24μ long. My specimens ranged from 42.5 to 75.3μ in length with a flagellum 32μ long. The measurements of the trypanosomes from newts 19 and 15 are particularly interesting. The data show that in *every one* of the trypanosomes from newt 19 the distance from the anterior end to the center of the nucleus is considerably greater than it is in *any* of the specimens from newt 15. This distance in newt 19 ranges from 31 to 44μ, whereas in newt 15 it ranges from 20 to 23μ. The average in the ten specimens from newt 19 is 36.3μ and in newt 15, 21.4μ, a difference of over 50 per cent. The differences between the distances from the center of the nucleus to the kinetonucleus are similar but not so great, those of the trypanosomes from newt 19 being only 25 per cent. greater than from newt 15. The distances from kinetonucleus to posterior end show differences averaging over 50 per cent., and the average difference in total length exclusive of the flagellum is approximately 50 per cent.

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* By the kinetonucleus is meant the body called by the French centrosome, by the Germans blepharoplast, and by certain Americans parabasal.

TABLE 1.—VARIATION IN LENGTH AND WIDTH OF 100 SPECIMENS OF *T. diemyctyli* TAKEN AT RANDOM, TEN FROM EACH OF TEN NEWTS, AND THE AVERAGE LENGTH AND WIDTH OF THE SAME GROUPS OF TEN. ALL MEASUREMENTS IN MICRONS

Number of Newt	Anterior End of Body to Center of Nucleus		Center of Nucleus to Kinetonucleus		Kinetonucleus to Posterior End		Total Length Exclusive of Flagellum		Width of Body at Nucleus	
	Variation	Average	Variation	Average	Variation	Average	Variation	Average	Variation	Average
19	31-44	36.3	23-32	25.6	5.6-6.9	6.1	61.6-75.3	68.0	3.1-3.8	3.3
20	32-42	35.5	23-29	26.1	5.0-6.9	5.9	58.6-72.9	66.5	2.5-4.4	3.3
14	28-33	30.5	26-29	27.7	3.1-5.6	4.3	59.4-66.6	62.5	2.5-3.1	2.9
18	23-36	27.9	22-27	23.9	3.8-5.0	4.3	50.8-65.4	56.1	2.5-3.8	2.9
16	22-31	27.2	20-30	23.9	3.1-5.6	3.9	45.1-65.4	55.0	2.5-3.1	2.6
10	23-31	26.4	21-26	23.9	3.8-4.4	4.3	48.8-60.0	54.6	3.1-4.4	3.4
17	21-27	24.1	22-25	24.0	3.1-3.8	3.5	48.1-55.8	51.6	2.5-3.1	2.7
12	23-29	25.1	21-25	22.9	2.5-3.8	3.3	38.1-57.1	51.3	2.5-3.8	3.0
9	19-29	24.4	18-27	21.8	3.1-4.4	3.6	45.0-60.3	49.7	1.9-3.1	2.6
15	29-23	21.4	19-22	20.6	2.2-3.4	2.7	42.5-48.4	44.7	1.9-2.5	2.2

The striking fact brought out by a comparison of these data is the large and constant difference in length between the two sets of trypanosomes taken from two different hosts of the same species. That this difference in length is not due to methods of preparation causing the elongation of one set and the contraction of the other is evident when a comparison is made of the diameters of the body in the region of the nucleus. The long specimens from newt 19 were also thicker than the short specimens from newt 15, the former averaging 3.3μ in diameter, and the latter only 2.2μ. The trypanosomes in newt 19 were therefore uniformly larger in all dimensions than those in newt 15.

Further study of Table 1 shows that the sets of 10 trypanosomes from each of the 10 newts were comparatively constant in their measurements. On the whole, the longest specimens are also the thickest, and length and width decrease together as one proceeds down the table.

In every set the average distance from the anterior end to the center of the nucleus is greater than that from the center of the nucleus to the kinetonucleus. When these distances are compared in the different sets, however, considerable variation becomes evident. For example, in specimens from newt 19 the difference between these two distances averages 10.7μ whereas in those from newt 17 the average difference is only 0.1μ and in those from newt 15 only 0.8μ. These distances are more uniform also in trypanosomes from newt 15 than in those from the other newts. The variations in the distances from the kinetonucleus to the posterior end were slight in the trypanosomes of each set, but the average distance is greatest in those of the longest set and becomes gradually less as the total length decreases.

After the work just described was completed it seemed desirable to obtain a more accurate measure of the differences between the trypanosomes in newts 19 and 15. Ninety more specimens from each newt were therefore measured by my assistant and the results are indicated in Table 2; this gives the averages of the various distances for the first ten trypanosomes measured from newts 19 and 15, for the succeeding ninety and for the entire one hundred. It is interesting to note that the averages for the first ten are very nearly the same as for the succeeding ninety. This indicates that the data obtained by measuring ten specimens from each newt as presented in Table 1 give fairly accurate averages.

Among the specimens from newt 15 six were found that were very much larger than any of the others. Measurements of these six are given in Table 2 and were omitted from those used for getting the averages of the 100 specimens given in this table. These six specimens resemble very closely those taken from newt 19 and apparently represent a type differing widely from the other more abundant trypanosomes from newt 15. Several explanations suggest themselves to account for the diversity between these two types found in a single host. Most probably there are here two size races of one species or there may be two distinct species living in a single host. The two types may possibly represent sexual stages of one species and although from what is known of the life cycles of other blood-inhabiting protozoa one would expect to find the sexual stages in the invertebrate host, still, as in the malarial organism, gametocytes may be developed in the vertebrate and remain dormant until stimulated to further activity within the invertebrate host. It is also possible that the two types of trypanosomes from a single host may be due to different stages of growth. A final suggestion is that *T. diemyctyli* is dimorphic.

DISCUSSION

No one has succeeded in classifying satisfactorily the trypanosomes and their allies, a condition due in part to the difficulty of determining morphologic differences of diagnostic value and to the fact that many species are polymorphic. *T. diemyctyli* is a favorable form for study because it is apparently monomorphic. Its life history, however, is unknown. The account Tobey gives of this species and the experience of the writer indicate that types other than the long, slender form do not occur in the blood of the newt, at least at the time of year when the examinations were made (May). No specimens were found that showed any signs of division and hence it seems safe to assume that all of the organisms measured represented "adult" forms.

TABLE 2.—AVERAGE LENGTH AND WIDTH IN MICRONS OF SPECIMENS OF *T. diemyctyli* FROM NEWTS 19 AND 15

	Anterior End of Body to Center of Nucleus	Center of Nucleus to Kineto-nucleus	Kineto-nucleus to Posterior End	Total Length Exclusive of Flagellum	Width of Body at Nucleus
Specimens from newt 19:					
Average of specimens 1-10.....	36.3	25.6	6.1	68.0	3.3
Average of specimens 11-100...	35.1	28.0	6.5	69.6	3.6
Average of specimens 1-100....	35.7	26.8	6.3	68.8	3.5
Specimens from newt 15:					
Average of specimens 1-10.....	21.4	20.6	2.7	44.7	2.2
Average of specimens 11-100...	21.9	21.4	2.3	45.7	2.9
Average of specimens 1-100....	21.7	21.0	2.5	45.2	2.6
Average of 6 largest specimens	33.5	26.3	4.8	64.6	3.5

Two hypotheses suggest themselves to account for the constant diversities in the total length and the length of portions of the trypanosomes from the different individual newts; (1) the observations may deal with pure lines, and (2) the organisms in one newt may be derived from various lines but may have become comparatively uniform in size due to life in one environment. The differences between groups of trypanosomes from different hosts might be accounted for by differences in the environment.

Pure Lines in Protozoa.—It has been shown by many investigators that "wild" specimens of free-living protozoa differ from one another in their heritable characteristics and that the descendants derived by vegetative reproduction from one "wild" individual may be uniformly different from those descended from another "wild" individual. The number of these pure lines that may exist in nature seems almost infinite.

Considerable interest has recently been created by the discovery of different strains of cysts of *Entamoeba histolytica* and *E. coli*. Mathis and Mecier (1916, 1917) recognize cysts of two sizes from *E. histolytica* which they consider indicates a sort of sexual dimorphism. Various strains of cysts as regards size have also been noted in *E. histolytica* by Wenyon and O'Connor (1917), Dobell and Jepps (1917), Matthews (1918), Mackinnon (1918), Smith (1918, 1919) and Kofoid, Kornhauser, and Swezy (1919). The evidence indicates the existence in these parasitic protozoa of heritably diverse races similar to those that have been described in a number of free-living protozoa. What influence environmental factors may have on the size of the cysts can be determined in several ways; for example, single specimens could be isolated from cultures and pure lines obtained from these also in culture. The effects of changes in environment could then be observed by modifying the culture medium or by inoculating specimens from the same pure line into different laboratory animals.

The habitat of the intestinal amoebae resembles that of the trypanosomes in certain respects although it may be more or less varied because of the many different kinds of food taken into the alimentary canal. The composition of the blood differs in different species of animals and to a lesser degree in different individuals of the same species. This means that trypanosomes also are subjected to differences in their environment. No one knows what effect these different environments may have on trypanosomes belonging to the same pure line, but as noted above a method of determining this point is available and is being put to the test in this laboratory.

SUMMARY

(1) Every one of 78 aquatic specimens and 2 of 7 land specimens of the newt, *Diemyctylus viridescens,* collected in Pennsylvania were found to be infected with *Trypanosoma diemyctyli* Tobey. No trypanosomes were found in 72 specimens of 5 other species of salamanders. Inoculation experiments with *T. diemyctyli* on 6 species of salamanders and 2 species of frogs were unsuccessful.

(2) Measurements were made of trypanosomes from 10 newts. These groups of trypanosomes differed from one another in their range of variation in total length exclusive of the flagellum, in the length of portions, and in the width of the body, in the average length of the entire body exclusive of the flagellum, in the average length of portions and in the average width.

(3) Length and width show a positive correlation and on an average the longer the specimen the wider it is.

(4) Of 106 trypanosomes from one newt, 100 were uniformly small and the remaining 6 were much larger, indicating 2 different types in a single host.

(5) The different types of trypanosomes obtained from the different newts are probably races of one species that are heritably diverse in size. They may, however, belong to different species or may be sexual phases of a single species, or may differ because of changes due to the environment.

LITERATURE CITED

Bruce, Sir David. 1914.—Classification of the African trypanosomes pathogenic to man and domestic animals. Trans. Soc. Trop. Med. and Hyg., 8 : 1-22.

Duke, H. L. 1912.—A camel trypanosome with some remarks on the biometric method of diagnosing trypanosomes. Proc. Roy. Soc., B, 85 : 563-568.

Pearson, K. 1914.—On the probability that the two independent distributions of frequency are really samples of the same population, etc. Biometrika, 10 : 85-143.

Robertson, Muriel. 1912.—Notes on the polymorphism of Trypanosoma gambiense in the blood and its relation to the exogenous cycle in Glossina palpalis. Proc. Roy. Soc., B, 85 : 527-539.

Stephens, J. W. W., and Fantham, H. B. 1912.—The measurement of Trypanosoma rhodesiense. Proc. Roy. Soc., B, 85 : 223-234.

Tobey, E. N. 1906.—Trypanosomata and trypanosomiasis. Jour. Med. Res., 10 : 117-146.

1906 a.—Trypanosomes in the newt. Jour. Med. Res., 10 : 147-148.

York, W., and Blacklock, B. 1914.—The differentiation of the more important trypanosomes. Ann. Trop. Med. and Parasit., 8 : 1-12.

References to other literature on the specific identification of trypanosomes may be found in the above papers, especially that by Pearson.

A NEW CYSTOPHOROUS CERCARIA FROM CALIFORNIA

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A NEW CYSTOPHOROUS CERCARIA FROM CALIFORNIA *

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A number of specimens of *Physa occidentalis* collected from Lake Temescal, a city reservoir near Oakland, California, were heavily infested with a new cercaria, to which the name *Cercaria californiensis* spec. nov. will be given. The infection was localized in the digestive gland of the snail. At the time that the examinations were made (September to December) there were found in the infected snails only rediae which contained cercariae. No sporocysts or rediae containing rediae were found.

The rediae of this species vary considerably in size, ranging from 0.5 mm. to 1.6 mm. in length and have a width equal to about one-fifth or one-fourth the length. They are elongate sacs, slightly narrowed at the anterior end, and are without lateral projections. The body walls of a few of these rediae contain a pale orange pigment, the others being quite transparent. Contrary to what is usually observed in pigmented parthenitae, the pigment in this species appears in the immature rediae, the older ones being always unpigmented. The pharynx is small and the intestine extends about one-fourth of the total length. No birth pore is present. When the rediae are first freed from the digestive gland of the snail host they contract and expand actively for a few minutes, but are unable to move from place to place. Cercariae and developing germ balls fill the body cavity of the rediae from the posterior end to the region just behind the pharynx. The number of fully developed cercariae contained within a redia varies from eight to fifty. This great variation in number can easily be explained since in the absence of a birth pore all the cercariae which develop must remain within the redia.

The body of *Cercaria californiensis* (Fig. 2) is very small, having a length varying from 0.12 mm. to 0.18 mm. according to the state of contraction. The attachment between the tail and the body is weak, and is usually broken soon after the cercaria is freed from the redia for study. While still attached to the tail the cercaria was never seen to move about, but when it had broken loose it could locomote slowly by the use of its suckers. The body cannot be contracted into the tail, but always keeps the relation to the tail shown in figure 1 while within the redia and, when freed from the redia, the position shown in figure 2. The size and position of the various organs of the body can be made out readily from figure 2. The ventral sucker is slightly larger than the oral and is in the posterior third of the body.

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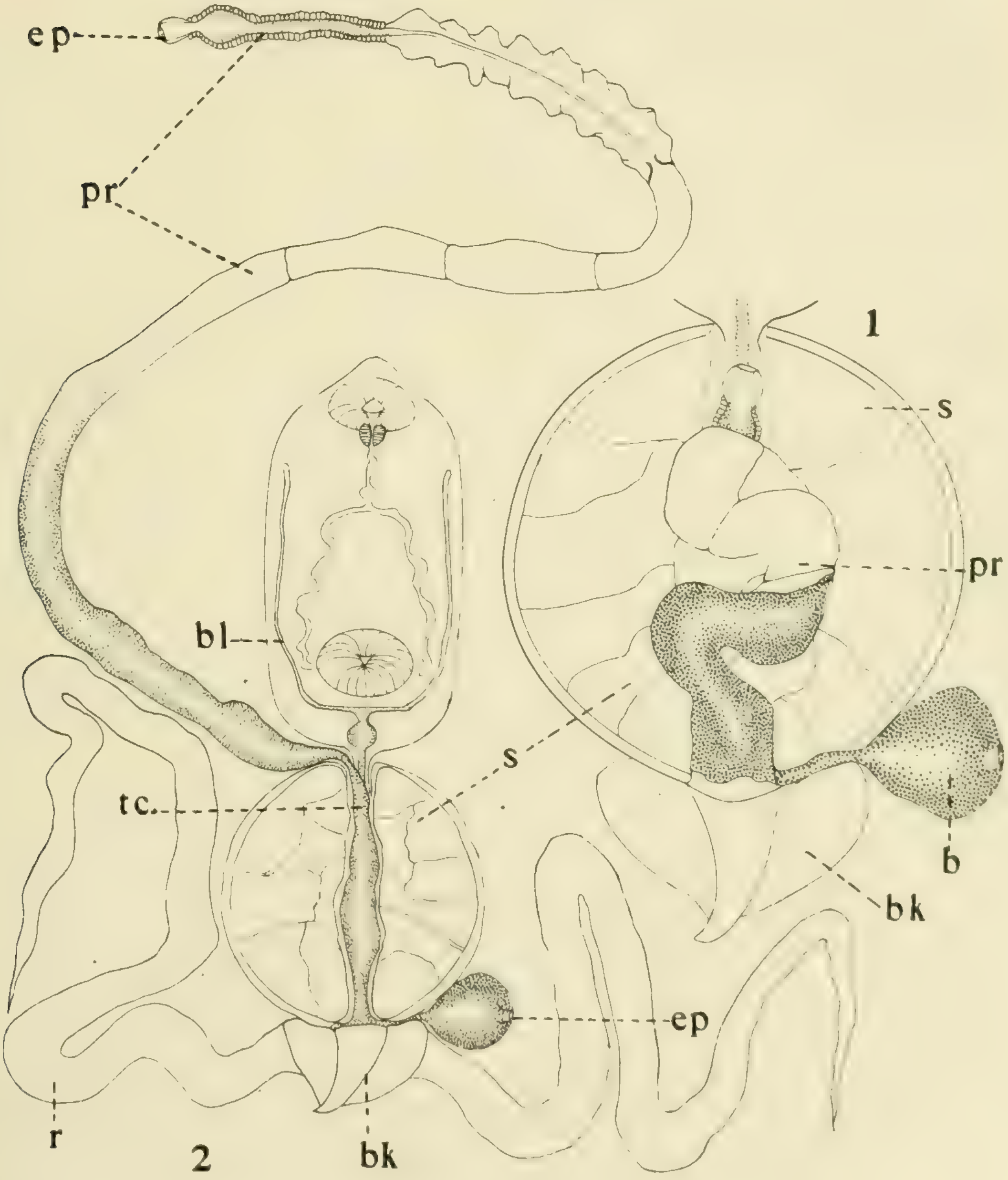
* A publication from the Department of Medical Zoology of the School of Hygiene and Public Health of Johns Hopkins University and from the Department of Zoology of the University of California.



The mouth of *C. californiensis* is on the ventral side a little distance back of the pointed anterior tip. There is no prepharynx.

The pharynx is small and is followed by an esophagus of medium length (Fig. 2). The intestinal ceca extend back to the level of the ventral sucker.

Of the excretory system only the parts of the bladder were distinguished (Fig. 2, *bl*). The undivided portion of the bladder has a globular enlargement containing the highly refractive concretions which are so comonly found in the excretory bladder of cercariae and agamodistomes. It is connected by a narrow tube with the excretory tubes of the tail. The bifurcations of the bladder were traced forward to a point just back of the pharynx. The region of the bifurcations nearest the enlargement and the tube leading into the tail also contain the concretions. The peculiar relations of the excretory canals in the tail will be discussed later.

The tail of *C. californiensis* is very remarkably differentiated. Figure 1 shows its appearance when the cercaria is within the redia or just after it has been forced out. In this condition the tail appears as a large firm cuticular sphere (Fig. 1, *s*) with two short projections at its posterior end (Fig. 1, *bk* and *b*). One of these projections has the form of a cuticular beak (*bk*). It is entirely transparent and is divided into a central area which is pointed posteriorly and two lateral areas. The beak is so transparent that nothing can be seen of the ribbons (Fig. 2, *r*) which are later extruded from the lateral areas. To one side of the beak is a bulb (Fig. 1, *b*) with an opening on its outer surface. This bulb is loaded with excretory concretions. The sphere has a thick cuticular wall and is very difficult to break when pressure is applied on the cover glass above it. The sphere has a central column of living material (Fig. 2, *tc*) along which is folded a very complex cylindrical structure. The portion of the sphere between the central column and the wall is highly vacuolated. The posterior region of this body is directly connected with the column of the sphere, and a tube from the excretory bladder of the body extends down into this column.

Soon after the cercaria is freed from the redia two flat transparent cuticular ribbons (Fig. 2, *r*) are extended out laterally from the sides of the beak. These ribbons are pointed and have no apparent structural differentiation. At the same time there is slowly protruded from the place where the body joins the tail sphere the long cylindrical projection which, in the condition indicated in figure 1, is folded along the column of the sphere (Fig. 1, *pr*). This structure, which will be called the excretory projection, is protruded until it has a length more than three times that of the body of the cercaria (Fig. 2, *pr*). The excretory projection is a hollow cuticular cylinder. The proximal half of this cylinder is undifferentiated, has a very thin wall and contains excretory concretions. Beyond the middle the excretory projection appears to be jointed externally. At about the beginning of the distal third of the excretory projection the cuticular wall becomes much thicker, narrowing the lumen. In the first half of this region the greatly thickened cuticula appears to be ruffled. Distal to this region the lumen widens slightly and the cuticula appears to be fluted. The excretory projection ends with a dilation followed by a cup-like structure with a large opening at its end. The first half of the excretory projection and the last fluted part contain excretory concretions. The excretory projection is broadly attached to the posterior part of the column of the tail (Fig. 2, *tc*). The differentiations of the excretory projection are shown in figure 2.

The presence of excretory concretions in various parts of the tail of *C. californiensis* indicates the relation of these parts to the excretory system. There are two openings of the excretory system in the tail (Fig. 2, *ep*), one on the outer surface of the excretory bulb, and the other at the end of the excretory projection. The excretory bulb is directly continuous with a tube which runs from the bladder of the body down the column of the tail. The lumen of the excretory projection is also directly connected with this tube. These connections are indicated by the presence of the concretions in the various structures. Movements of the concretions could be followed from the tube in the column of the tail into the excretory bulb. Excreta from the body of *C. californiensis* can escape after passing down the column of the tail-sphere either by the pore of the excretory bulb or after passing along the excretory projection from the opening at its end.

Apart from the relation to the excretory system described above, it is difficult to suggest functions for the various parts of the tail of *C. californiensis*. The function of the tail of a cercaria is evidently to aid it in that period of activity, usually including a short period of free life, from the time it leaves its parthenita until it is settled in its secondary intermediate host, its final host, or is encysted in the open waiting to be taken into its final host. We have no direct evidence in regard to the further development or activities of *C. californiensis*. There are certain things, however, which it is perfectly evident that this cercaria does not do. The absence of a stylet and cephalic glands and the weak muscular development of the body of the cercaria make it evident that it does not penetrate actively into the next host. The absence of cystogenous glands in the body and the inability to withdraw into the vesicle of the tail show that it does not encyst in the open and wait to be taken into its next host. Finally the fact that the redia has no birth pore, that the cercariae accumulate in the redia and that they are practically incapable of locomotion in the open, makes it very probable that this cercaria never leaves its intermediate host and continues its development in some vertebrate which feeds on this host.

There is nothing in such a life cycle which would make it possible to explain the complex structure of the tail of *C. californiensis* in terms of function. The fact that in three closely related forms *C. cystophora* (Wagener, 1866), *C. sagittarius* (Ssinitzin, 1911: 15-19), and *C. yoshidae** (Yoshida, 1917) the body of the cercaria can be withdrawn into the tail for protection suggests that this is the primary function of such a vesicle as the sphere of *C. californiensis*. A change in life cycle which eliminated the free stage and therefore the importance of this function may have led to the loss of the power of withdrawal into the tail vesicle without the loss of that structure. It is still more difficult to determine any function for the appendages of the tail. Ssinitsin, (1911: 18) suggests that the various appendages of the tail of *C. sagittarius* hold the tail vesicle, which forms a cyst containing the cercaria, in position in the digestive tract of the final host until the cercaria has had a chance to get out of its cyst. Such a function for the excretory projection and ribbons of *C. californiensis* seems very improbable since in the first place this cercaria does not become encysted in the vesicle of the tail and in the second place the body is so weakly attached to the tail that even a slight pull will break the connection. It is, of course, possible that these appendages may also represent structures which have lost their function through modifications of the life cycle. Finally it is very probable that structures may develop in connection with the evolution of such a diversified organ as the cercaria tail which have no function whatsoever.

C. californiensis belongs to a small group of cercariae which Ssinitsin (1911: 20) named the Cystophrous Cercariae. He characterizes this group as containing cercaria which possess a vesicular tail with various appendages. The first cercaria of this type to be described was *Cercaria cystophora* which Wagener (1866) reported from *Planorbis marginatus*. Another cercaria which probably belongs to this group was described by Sonsino (1892) from *Cleopatra bulimoides* as *C. capsularis*. Sonsino's original description is hardly sufficiently detailed to make the relationship of this species clear. The same cercaria is later described in the immature state by Looss (1896). These two descriptions taken together make it pretty certain that this form belongs to the cystophorous cercariae, but do not give enough data to make a detailed comparison possible. Pelseneer (1906) described two marine representatives of this group, *C. appendiculata* from *Natica alderi* and *C. vaullegeardi* from *Trochus cinerarius.* Later Ssinitzin (1911: 15-21) described two more marine cystophorous cercariae, *C. sagittarius* from *Cerithialum exille* and *C. laqueator* from *Rissoa venusta.* Finally there should be added to this group *C. yoshidae* (Yoshida, 1917) from *Melania libertina* from Japan and *C. californiensis.* While the cystophorous cercariae vary considerably in structural details, they have so many important points in common, that we consider that they form a natural group.

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* I use this name to designate the species which Yoshida describes as Cercaria F.

All the cystophorous cercariae except *C. vaullegeardi* (Pelseneer) develop in rediae. Mother sporocysts in which the rediae develop have been described for two species, *C. sagittarius* Ssinitzin (1911: 15) and *C. cystophora* (Wagener, 1866). In none of the species were rediae which contained rediae found and the descriptions of the two species of which the mother sporocysts were found, indicates that such a stage is not present in the life histories of the members of this group. The rediae are much alike having no locomotor appendages, a very small pharynx and a voluminous digestive tract varying from one-fourth the length of the redia in *C. californiensis* to two-thirds its length in *C. yoshidae.*

The body of the cercariae in all cystophorous cercariae is very small and the primordial adult characters are very slightly developed. This is indicated by the small size, the lack of clear differentiation of the organs and the short distance between the ventral sucker and the posterior end. The bodies of these cercariae lack entirely all adaptive larval structures for penetration and encystment, i. e., stylet, cephalic glands and cystogenous glands. The digestive system in those forms for which it has been described is much like that of *C. californiensis* (Fig. 1), except that the length of the intestinal ceca varies. The excretory bladder is y-shaped.

The tails in these forms differ considerably in detail, but are all built on the same fundamental plan. There is present in each species a central vesicle or sphere as we have called it in *C. californiensis.* This is a firm structure with a thick cuticular wall which was evidently developed to function as a cyst into which the cercaria can be withdrawn into the cavity of the sphere as in *C. sagittarius, C. cystophora, C. yoshidae.* In regard to *C. laqueator* and *C. californiensis* the statement is made that the body of the cercaria cannot be withdrawn into the vesicle of the tail. It is evident that this ability of the cercaria to withdraw into the tail would be a very important protective measure at the time of entrance of the cercaria into its next host. Besides the central vesicle there is found in every species of this group a protractile appendage comparable to the structure we have called the excretory projection in *C. californiensis.* This structure differs greatly in the various species and in some is not sufficiently described for comparison. The various other appendages which are found on the tails of the Cystophorous cercariae are so remarkably varied and grotesque that it is not only impossible to get an idea of what their function may be, but also there seems to be no homologies which can be worked out between them.

Further development is known for only one of the cystophorous cercariae, *C. cystophora,* which develops into *Halipegus ovicaudatus* which is found in the mouth cavity of the European frog. Ssinitzin (1905 and 1907) finds the agamodistine stage of this species free in the body cavity of the nymph of the dragon-fly *Calopteryx virgo.* On this account he suggests (Ssinitzin, 1911:21) that the life cycles of the other members of this group will probably follow the same course. This deduction does not seem to us to necessarily follow since a fundamental modification of life cycle might come to a species, and yet the structural adaptations for the previous mode of life might be retained. Certainly in the case of *C. californiensis* there could hardly be a secondary intermediate host, since it seems very improbable that this cercaria ever leaves its molluscan intermediary.

SUMMARY

1. *Cercaria californiensis,* a new species of cercaria from *Physa occidentalis,* has a very small body which shows no adaptive larval characters and but slight development of primordial adult characters.

2. The tail of this species is very remarkably differentiated, consisting of a central cuticular vesicle, the sphere, and various projections and appendages.

3. It is not possible to explain the functions of the parts of this tail in terms of activities of the cercaria since the cercaria probably never leaves the redia within its intermediate host.

4. *Cercaria californiensis* belongs to a natural group, the cystophorous cercariae which are characterized by a small, very slightly differentiated body and an extremely complex tail consisting of a central cuticular vesicle, with various appendages.

LITERATURE CITED

Looss, A. 1896.—Recherches sur la faune parasitaire de l' Egypte. Premiére partie. Mem. l'institute Egyptien, 3:1-252; 16 pls.

Pelseneer, P. 1906.—Trematodes parasites de mollusques marins. Bull. scien. France et Belg., 40:161-186; 5 pls.

Sonsino, P. 1892.—Studi sui parassiti molluschi di acqua dolce dintorni di Cairo in Egitto. Leuckart's Festschr., pp. 134-147.

Ssinitzin, D. Th. 1905.—Studies on the life-cycle of trematodes. The Distomes of the Fish and Frogs in the Environs of Warsaw. (Russian.) Mem. soc. nat. Varsovie biol., 15:1-210, 6 pls.

1907.—Observations zur les métamorphoses des Trématodes. Arch. zool. expér et géner., 37, notes et rev., pp. 21-27.

1911.—The Parthenogenetic Generation of Trematodes and their Progeny in the Molluscs of the Black Sea. (Russian) Records Imp. Acad. Sci., (8) 30:1-127; 6 pls.

Wagener, G. R. 1866.—Ueber Redien und Sporocysten Filippi. Arch. Anat., Physiol., u. wissensch. Med., 145-150. 6 pls.

Yoshida, S. 1917.—On the Cercariae from Melania in Japan. (Japanese) Dobutsu Jaku Zasshi (Zoological Magazine) vol. 29, no. 342.

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THE DEVELOPMENT OF THE JAPANESE BLOOD-FLUKE, *SCHISTOSOMA JAPONICUM* KATSURADA, IN ITS FINAL HOST.*

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PLATES I TO IV.

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I. COMPARISON OF THE CERCARIA OF *Schistosoma japonicum* WITH THE ADULT.

Since the discovery of the intermediate host of *Schistosoma japonicum* by Miyairi and Suzuki in 1913, our knowledge of the life-cycle of the human blood-flukes has been very greatly increased. Very little is known, however, of the development after the cercaria enters the final host. The studies recorded in this paper were undertaken with the hope of filling in, to some extent, this gap in our knowledge in the case of *Schistosoma japonicum*. In order to point out the problems involved in this investigation, I will make a brief comparison between the cercaria of this species and the adult.

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* A contribution from the Department of Medical Zoölogy of the School of Hygiene and Public Health of the Johns Hopkins University.

The cercaria of *S. japonicum* (Text Figs. 1 and 2) * is very highly specialized for that critical period of intense activity from the time that it leaves the shelter of its snail intermediate host until it has penetrated safely through the skin of its final mammalian host.

During this period two demands are made upon the activities of the cercaria. One of these is for locomotion, both freely in the water and on a surface. For this activity the cercaria has a well developed muscular system, a strong tail, a ventral sucker which can be firmly attached, and a method by which it can take hold with its anterior end. Details of the methods of locomotion of the cercaria of *S. japonicum* have been reported in a previous paper (Cort, 1919, 502–505). The second demand that the cercaria must be prepared to meet is that of penetration through the thick skin of the mammalian host. The whole structure and activity of the cercaria is modified profoundly for this activity (Cort, 1919, 496–502). In fact, it has become essentially a mechanism for skin penetration. There have been developed very large cephalic glands (Text Figs. 1 and 2, *cg*) for dissolving tissue. The musculature of the body, and especially of the oral suckers, is definitely adapted for penetration, and the arrangement of the spines is an admirable adaptation for this purpose. This unusual development of the adaptive larval characters, especially the enormous size of the cephalic glands, has kept in a very undeveloped condition the primordial adult characters. The extreme immaturity of the cercaria is indicated by its small size, the slight development of its postacetabular region and the very rudimentary character of its nervous, excretory, digestive and reproductive systems

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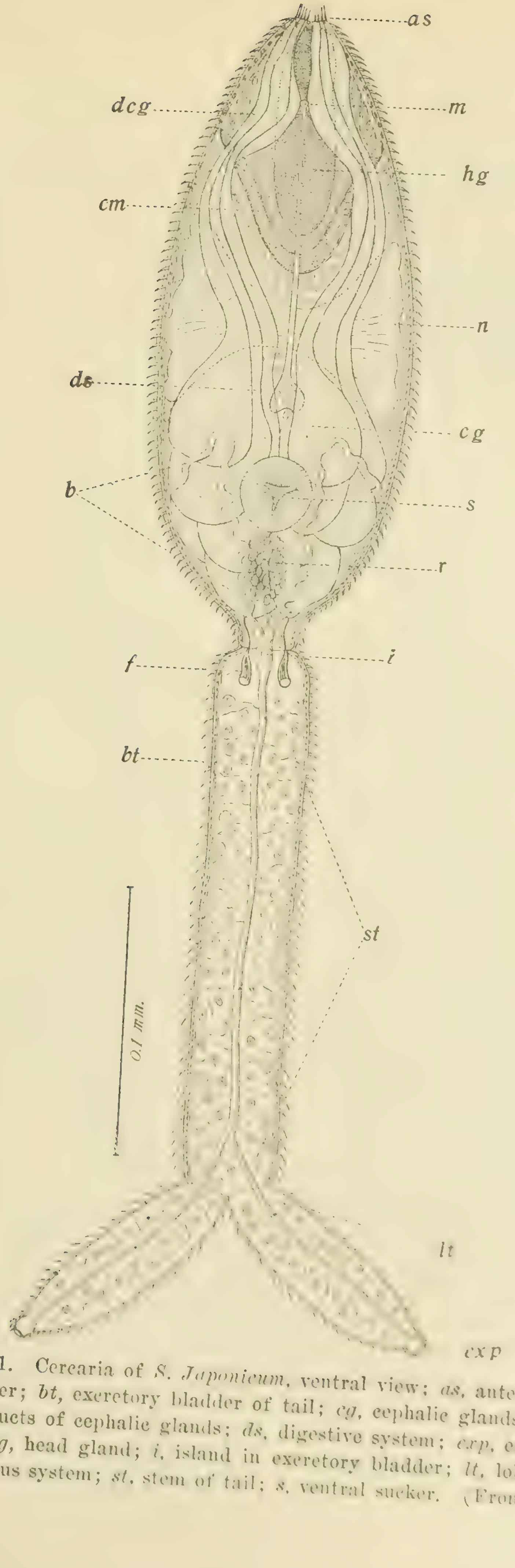
* In my paper on the cercaria of *S. japonicum* (Cort, 1919, 501) I described and figured forward pointing spines around the openings of the cephalic glands, which I believed aided the cercaria in penetration. Faust (1919, 168, Figs. 4 and 5) demonstrated in the cercaria of *S. mansoni* and related forms, that these spines on the anterior end of schistosome cercariae are hollow in the center, cap the openings of the cephalic glands, and that the secretions of these glands pour through the hollow spines. I have been able to confirm Faust's observations on the cercaria of *S. japonicum*.



TEXT FIG. 1. Cercaria of *S. Japonicum*, ventral view; *as*, anterior spines; *b*, excretory bladder; *bt*, excretory bladder of tail; *cg*, cephalic glands; *cm*, circular muscles; *dcg*, ducts of cephalic glands; *ds*, digestive system; *exp*, excretory pore; *f*, flame cell; *hg*, head gland; *i*, island in excretory bladder; *lt*, lobe of tail; *m*, mouth; *n*, nervous system; *st*, stem of tail; *s*, ventral sucker. (From Cort, 1919.)

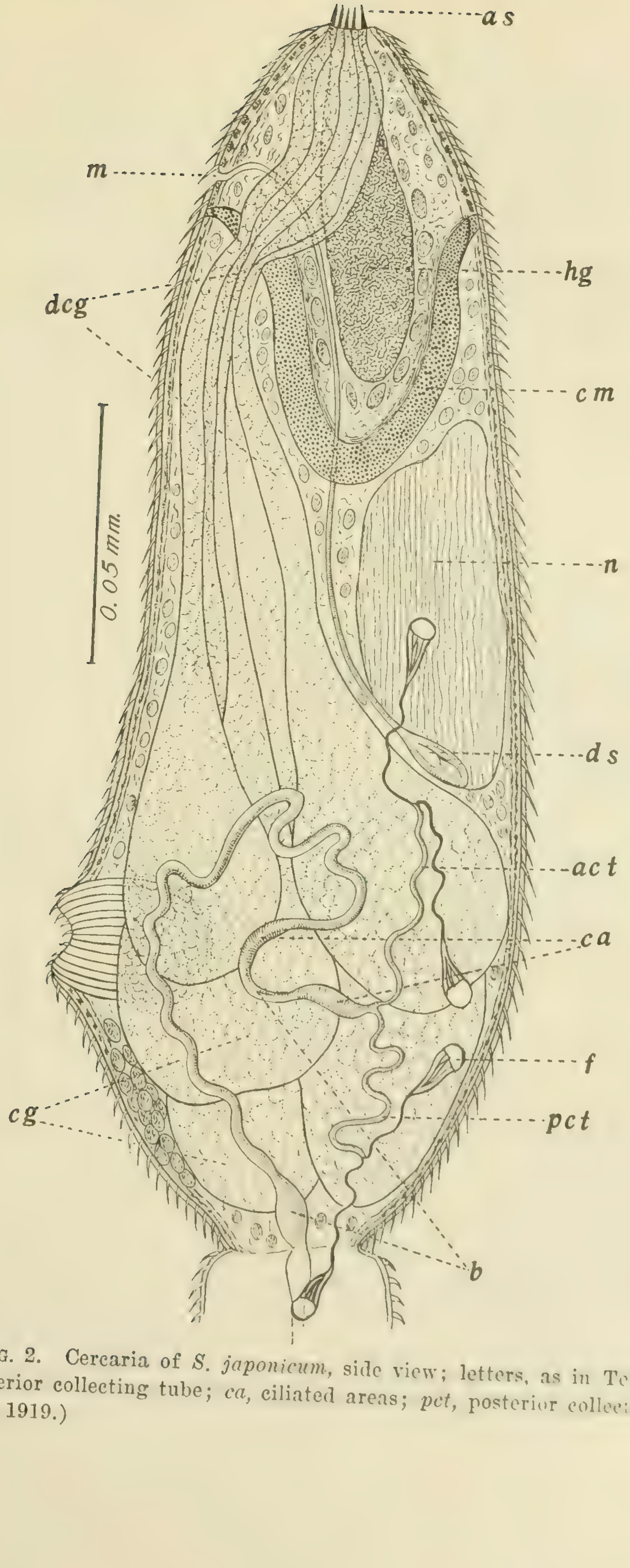
(Text Figs. 1 and 2). Finally, the sexual dimorphism which is such a striking characteristic of the adult has not been demonstrated in the cercaria.

The contrast in structure between the cercariae and the adults of *S. japonicum* (Figs. 11 (F) and 19 (M) is so striking that practically no similarity whatever can be made out between the various organs, unless the intermediate stages in development are known. The structure of the adult of this species is dominated by sexual dimorphism and adaptations for reproduction. The shape of the male is fundamentally modified from the general distome condition by the development of the gynaecophoric canal for holding the female in copulation. The female, on the other hand, has a very elongate, narrow body. These modifications have come about chiefly by changes in the postacetabular region of the body which are correlated with the gradual development of the reproductive organs.

The morphological changes which are necessary to produce the adult of *S. japonicum* from the cercaria are truly remarkable. The highly developed adaptive larval characters are lost soon after penetration. The length of the body must be increased fifty to even over one hundred times. The nervous and excretory systems already functioning in the cercaria must be greatly extended to meet the requirements of this rapid increase in size. The digestive system which is not yet functional in the cercaria must be organized for its activities and greatly increased in length, to keep pace with the extending body. The whole complexity of the reproductive system must be gradually evolved from the small group of nuclei, which are the primordia of this system in the cercaria (Text Figs. 1, *r*), in some individuals becoming male organs and in others female. Finally, the development of the secondary sexual characteristics which differentiate the sexes early, and later produce extreme sexual dimorphism, goes hand in hand with the development of the reproductive systems.

II. Material and technique.

The problems outlined above are interesting not only, because the changes from the cercaria to the adult in *Schistosoma japonicum* go on in the human body, but also from the more general biological viewpoint, since our knowledge is very inadequate of this stage in the development of the whole group of digenetic trematodes. An opportunity to attack this problem presented itself when on June



TEXT FIG. 2. Cercaria of *S. japonicum*, side view; letters, as in Text fig. 1; also *act*, anterior collecting tube; *ca*, ciliated areas; *pct*, posterior collecting tube. (From Cort, 1919.)

23, 1919, I received from Dr. Sadao Yoshida of the Osaka Medical College, a package containing a large number of dried specimens of *Blanfordia nosophora* (Robson), the intermediate host of *S. japonicum*. These snails had been collected on May 28 from an area where Japanese schistosomiasis is prevalent. About 85 per cent. of them became active when placed in water, and about 4 per cent. of the living snails were found to harbor the cercariae of *S. japonicum.*

During the evening of the same day on which this material was received, a tablespoonful of the dried snails was placed in each of three small glass aquaria in water, at a depth of about two and one half inches. On the following day, it was found that almost all of the snails had become active and that the water was swarming with cercariae.

At 4 P. M. of this day, June 24th, thirteen white mice were placed in the three aquaria. The depth of the water was such that they could rest their hind legs on the bottom and just keep their heads above the surface of the water. The mice were kept in the water about twenty-five minutes. The following morning, seventeen hours after the first exposure, the mice were again placed for about twenty minutes in water, containing the active cercariae from four infected snails which had been crushed.

The first of the mice was examined forty hours after the first exposure to infection, and three young worms were found in the blood. The other mice were examined on the tenth, twelfth, fourteenth, sixteenth, eighteenth, nineteenth and twenty-first days after the first exposure. In all these mice large numbers of the worms were found in the veins of the liver. In fact, the mice were so heavily infected that after the sixteenth day they began to die, and it was barely possible to keep the last one alive until after the twenty-first day. In addition to the material from these thirteen mice, there was also available for comparison a number of specimens taken from mice thirty days after infection.

To recover the young worms the livers of the mice were carefully teased apart in normal saline, and the worms picked out with a small pipette. In the earlier examinations it was necessary to search carefully with a microscope to find the young schistosomes. On the later days they had grown to such an extent that they could be seen with the naked eye. The worms were killed and fixed by pouring suddenly over them a hot saturated aqueous solution of corrosive sublimate containing 2 per cent. of acetic acid. This method proved satisfactory since it caught the worms well extended. My observations were made from toto mounts prepared from this material.

The material available for study gave me a series of developmental stages from my smallest form, from the veins of the liver, 0.24 mm. in length, up to sexual maturity and copulation. A detailed study of that interesting period of reorganization from the cercaria to this smallest form will have to be left for future investigation. Since my studies were made on preserved material I have no data on the development of the excretory system. The present paper then includes a study of the changes in size and shape of the body, the changes in the suckers, the growth of the digestive system and the development of the reproductive organs and the secondary sexual characters which occurred in the veins of the livers of my experimental mice.

In addition to the data from my studies of the material discussed above, I shall include in this paper an account of all the phases in the life cycle from the free cercaria to maturity. This includes a discussion of the question of the time of determination of sex and possible sexual dimorphism in the cercaria, the method of penetration into the final host, the course of migration in the final host, and the early stages of development not included in my own studies. Most of the literature on these subjects is in the Japanese language. These publications were made available to me through the kind help of Dr. S. Yokogawa, of the Medical College of Formosa, and of Mr. Ibara, a graduate student in Johns Hopkins University who translated for me a part of Miyagawa's 1916 paper. I wish also to thank Miss Ethel Norris for her careful work in the preparation of the drawings included in this paper.

III. The determination of sex and the beginning of sexual dimorphism.

Since sexual dimorphism can be seen at a very early stage in the development of the larval schistosome in the final host, it seems perfectly evident that sexual differentiation is already present in the cercaria stage. From this consideration the question naturally arises whether there is not really present a sexual dimorphism in the cercaria stage. Altho many workers have made studies and measurements of the human schistosomes no one has reported such differences. I have myself, examined a number of cercariae of *S. japonicum* with this point especially in mind, without noting any dimorphism. Dr. S. Yokogawa also informs me that he has made an extensive series of examinations and measurements of this cercaria in an attempt to find sexual differences, without success. Since the cercariae of the human schistosomes are very small and can extend and contract their bodies to an unusual extent, slight size differences might escape notice in the living specimens and be difficult, if not impossible, to detect in measurements of preserved material. Further, these studies have been made from cercariae obtained from infected snails which had been crushed to free the cercariae. Such material would contain various stages in the development. Therefore variations in size, due to differences in the developmental stages of the specimens studied, might obscure a slight sexual dimorphism. I believe that if careful measurements were made of large numbers of cercariae after their escape from the snails, it might be possible to demonstrate size dimorphism between the sexes of this species.

Recently, in some studies on a species of schistosome cercaria with eyespots, from *Planorbis trivolvis*, from Douglas Lake, Michigan, I have been able to demonstrate two distinct size types. This difference in size came to my attention first when I found that the curve plotted from the measurements of the cercariae which had escaped from a number of infested snails was distinctly bimodal. More extensive studies showed that the cercariae of this species fell into two distinct size groups. I further found from measurements of the cercariae from eleven infested snails that in the cercariae coming from a single snail only one of the size types was represented. The difference in size was so great betwen these two types that it could be recognized with the naked eye, when free swimming cercariae of the two types were placed in separate bottles. Measurements of the length of the body of the larger type showed a range of variation from 0.234 mm. to 0.28 mm., while in the smaller type, the range was from 0.207 mm. to 0.24 mm. Other measurements of the body and tail, which in this species is unusually large, showed like differences. The adult into which this cercaria develops is not known, although unsuccessful attempts were made to introduce it into ducks and rats. An analysis of its structure, however, places it near to the human schistosomes, in the family Schistosomidae. This relationship means that in all probability in the adult stage of this species the sexes are separate. I therefore, interpret the size differences in this species of cercaria as a sexual dimorphism. If this view is accepted, the fact that in one infested snail only one of the types of cercaria is represented, immediately becomes very significant. A more detailed account of the dimorphism of this species of cercaria will be published later in connection with a study of its structure and activities.

In this connection must also be cited the work of Tanabe (1919) on *Schistosoma japonicum*. This author found that in twenty-six out of thirty-one cases, when the cercariae from a single snail were used in infecting experimental animals, all the individuals developed were of the same sex. Dr. S. Yokogawa has given me permission to use in this connection, the results of some of his unpublished experiments along this line, which were performed several years ago. He found that when a cat, dog, or rabbit was infected with the cercariae from a single snail that worms of only one sex would develop. He also found that in these cases the worms would not develop to maturity. These two workers have developed independently the same hypothesis to explain the results of these experiments.

According to this hypothesis sex in the schistosomes is determined in the fertilized egg, and all the cercariae coming from a single miracidium are of the same sex. When all the individuals derived from the cercariae from a single snail were of the same sex, it would follow that the infestation in this snail was from a single miracidium or two or more miracidia of the same sex. In those cases where both sexes came from the same snail, this snail must have been originally infected with two or more miracidia representing both sexes. Now my findings recorded above in regard to dimorphism in a species of schistosome cercaria, and the presence in one snail of only one of these types lends further support to this hypothesis. Further, since in the life cycle of *S. japonicum*, the miracidium and the mother sporocyst are the only stages derived from a fertilized egg, it is in these stages that sex differentiation would theoretically be expected. Up to the present time, however, no one has examined these stages to determine whether they show a sexual dimorphism.

IV. PENETRATION OF THE CERCARIAE THROUGH THE SKIN OF THE FINAL HOST.

The structures of the body of the cercaria of *S. japonicum* which make possible the penetration through the thick skin of the mammalian host have been discussed above. It has also been clearly established (Fujinami and Nakamura, 1909; Katsurada and Hasegawa, 1910; Matsuura, 1909 and Fujinami, 1914) that infection with *S. japonicum* is produced by pentration of the cercariae through the skin or mucous membrane. Infection by way of the digestive tract has been shown to be impossible (Tsuchiya, 1910; Fujinami and Nakamura, 1911; and Miyagawa, 1912a), since the cercariae cannot resist the action of the digestive juices. However, the infection with drinking water is possible, since the cercariae taken into the mouth may penetrate through the mucous membrane of the mouth and pharynx. Penetration through the skin of the cercaria of *S. japonicum* has been discussed most fully by Narabayashi (1914, 1916). He finds that the cercariae can penetrate through wet skin at any point where they become attached, although they actually seem to enter most frequently by way of the hair follicles. Usually, they entered singly, altho sometimes two or three were found at the same place. In examinations of the skin from one to twenty hours after exposure to infection, the cercariae were found in the epidermis, dermis and subcuticula and sometimes in the deeper layers. The worms found in the skin tissues show various shapes and degrees of contraction. Usually, the tail is lost at the time of penetration through the skin, but in some cases it may be carried into the subcuticular layer. In one of my experimental mice I found a cercaria in the blood from the heart, with the tail still attached. The worms entering the skin seem to be attracted to the blood vessels, for soon numbers of them will be found in the capillaries, veins or lymph spaces of the dermis and subcuticula.

V. THE COURSE OF MIGRATION IN THE FINAL HOST.

The course which the larval blood flukes pursue in making their way from the skin to the blood vessels of the liver has been a matter of considerable controversy. Fujinami and Nakamura (1910) found the young parasites in the portal vein of a rabbit, three days after exposure to infection, and suggested that the course of migration in the body was probably by way of the blood stream. Miyagawa (1912; 1913; 1913a; 1913b) found the young flukes in the femoral and saphenous veins, in the hilus of the lymph glands, in the thoracic duct, and finally in the heart of experimental dogs. Later this same author (Miyagawa, 1916) also found the parasites in the capillary vessels of the lungs, kidney and intestinal walls, and in the left side of the heart. From his studies, he came to the conclusion that the young blood flukes might travel to the right side of the heart either by the veins or lymph vessels, be pumped into the lungs, pass the capillaries of the lungs and return to the heart. From the left side of the heart they would be carried into the systemic circulation, and must again pass through capillaries to reach the mesenteric veins and the portal vein. Narabayashi (1914, 1916) agreed with Miyagawa that the above course is sometimes followed by the worms. He concluded, however, that the majority of the young blood flukes after reaching the lungs leave the blood vessels and migrate to the liver through the tissues. Strong evidence for this course has been furnished by the extended researches of Narabayashi (1916). This author infected large numbers of very young mice with the cercariae of *S. japonicum* and made serial sections of their whole bodies. He found that the majority of the cercariae, after passing through the skin, entered the veins and that only a few reached the heart by way of the lymph vessels, since most of those that got into the lymphatic system died in the lymph glands. He found them only rarely in the capillaries of the lungs, in the left heart and in the blood vessels of the kidneys. The larger number he found migrating through the connective tissue of the posterior mediastinum, along the large blood vessels and nerve trunks, while a smaller number was encountered also in the anterior mediastinum. Still others were found in the pleural cavity. He also found them in the tissues of the diaphragm and on the surface of the liver, but never in the hepatic, splenic and mesenteric arteries. Recently Suyeyasa (1919) repeated Narabayashi's experiments and came to the same conclusions. It seems probable, therefore, from these studies, that the usual course of migration is for the larval blood flukes to leave the blood vessels of the lungs, to pass through the diaphragm, and to enter the liver from its surface.

It is probable from the character of the migratory course described above that there would be considerable variation in the time necessary for the migration. It would be expected, therefore, that in the course of a single infection larvae would keep entering the liver for a considerable length of time. That this actually happens is suggested by my finding very small larvae, less than 0.3 mm. in length, in the liver ten, twelve, and up to eighteen days after exposure to infection. This point will be discussed more in detail in a later section.

VI. THE MORPHOLOGICAL CHANGES IN THE DEVELOPMENT OF *S. japonicum* IN ITS FINAL HOST.

1. *Historical account.*

Up to the present time a detailed analysis of the morphological changes from the schistosome cercaria to the adult has not been made.

I find, however, that a number of authors have made observations on this stage of development. Most of these observations have been made on the development of *S. japonicum,* and most of them are included in papers written in Japanese. Leiper (1915) has figured a series of stages in the development of *S. haematobium* in its final host, but he does not describe or compare his stages, nor does he distinguish the sexes. Lutz (1919) in his discussion of *S. mansoni* gives very few data on this stage in development.

I will not attempt to discuss in detail all the observations which have been made on this stage in *S. japonicum,* but merely bring out the significant points in the studies of the various investigators. Miyagawa (1916) in his part of the Japanese monograph on *S. japonicum* (Fujinami, Tsuchiya and Miyagawa, 1916) discusses in some detail the work which has been done on this stage of development of *S. japonicum.* My discussion of the Japanese work is based upon reviews, upon a translation of the part of Miyagawa's paper dealing with this subject and upon discussions with Dr. S. Yokogawa.

Miyagawa (1916, 17) stated that in the larvae examined after entering the skin the "head gland" was found to be reduced in size, pressed to one side, and that later it disappeared. He also notes that in such worms the contents of the cephalic glands are much reduced in amount, and that these organs soon disappear.

No detailed morphological studies have been made of the development of the worms in the tissues, or during their migration to the liver. Miyagawa finds that the size of the young worms taken from the skin and the peripheral veins varies from 0.038 to 0.15 mm. in length, and from 0.012 to 0.068 mm. in width. Since the smaller limits of his ranges of variation are only a fraction of the size of the cercaria itself, it is evident that some of the specimens which he studied must have been extremely contracted, on account of the technique used in preservation. Narabayashi (1914) found young blood flukes in the skin having a length of 0.143 mm. and a width of 0.034 mm. Fujinami and Nakamura (1910) found a young fluke in the portal vein of a rabbit, three days after infection, with a length of 0.15 mm. and a width of 0.037 mm. Miyagawa (1916) also reports a worm in the portal vein of a dog, five days after exposure to infection, which measured 0.5 mm. in length and 0.058 mm. in width. Katsurada (1914, 372, Fig. 2) describes and figures a young stage 0.438 mm. in length and 0.12 mm. in width, taken from the liver of a dog on the seventh day after infection. This figure is evidently of a male of about the stage of my Fig. 3 (*M*). The oral sucker in this specimen had become definitely rounded off and the intestinal ceca are well developed, and distended with a dark brown material, altho not yet united. Miyagawa (1916) also notes that the spines in the young worms up to 0.3 mm. can be very clearly distinguished, but that it is very difficult to see the spines, even in the preacetabular region in a specimen 1 mm. in length. He further records that at the 0.3 mm. stage the oral sucker has changed from the peculiar condition of the cercaria to a well rounded muscular organ, with a good sized mouth cavity.

Miyagawa (1916) also gives some interesting information on the early development of the digestive system. He finds that in the larvae taken from the skin the digestive system is already functioning, since it can be seen clearly and is distended with dark staining material. In the early stages it appears like a distended bag which is connected with the oral sucker by the narrow esophagus. A little later, the intestinal ceca develop, and in forms 0.2 to 0.3 mm. in length, the shape is like a horse shoe. A little later the ceca unite, and the common portion reaches almost to the posterior end of the body. Miyagawa offers a very interesting observation on the relation of the liver to the development of the blood fluke. He differs from Narabayashi's (1916) position that the liver is simply a station in the course of migration of the worms, and believes that it is the natural location for the worms and that only in it can growth go on normally. Further, it is only when females deposit eggs that the copulating worms work their way out from the blood vessels of the liver to the mesenteric veins.

According to the Japanese workers the reproductive organs become differentiated late, but sexual dimorphism appears early. Fujinami and Nakamura (1911) were able to distinguish the sexes in specimens 0.5 to 0.7 mm. in length, which had developed in dogs. According to these authors, the body of the male becomes early broader than the female, especially in the region of the branching of the intestinal ceca. Also, the suckers are somewhat larger in the male than in the female. They further find that there is a distinct difference in the region where the intestinal ceca unite. In the male the region between the intestinal ceca, just in front of the point of union is a long narrow triangle, while in the female this region, where the ovary develops is even at an early stage wider. Miyagawa (1916) notes that the differentiation of the reproductive organs comes comparatively late, and that they can not be clearly distinguished until a length of about 1 mm. is reached. He finds copulating worms twenty days after infection.

2. *Growth in size.*

One of the most striking changes in the development of *S. japonicum* in its final host is the remarkable increase in size. In the published records the sizes given for the body of the cercariae vary from 0.09 mm. to 0.23 mm. These variations for the most part depend on whether the measurements were made from living or preserved specimens, on the state of contraction, and on the method of preservation used. Since the body of a well extended, preserved specimen measures about 0.15 mm., I will use this size for comparison of the cercaria with further stages of development. The largest size which I have seen recorded for the adult of *S. japonicum* was 26 mm. in length for the female, and 22.5 mm. for the male. These measurements were the maximum found by Tsuchiya for living specimens, taken from man, and by Katsurada (see Katsurada, 1914, 366) from living specimens 21 months old, taken from a dog. To attain this size an increase in length of about 130 times over the cercaria is necessary.

The growth of *S. japonicum* in the final host may be divided into three periods, i.e. (1) from the penetration of the cercaria until the young worm reaches the blood vessels of the liver, (2) from the time of reaching the liver until sexual maturity, and (3) from sexual maturity to maximum size.

There is but little increase in size in the young worm which has not yet reached the liver. The three specimens which I found in the blood of the first mouse, examined forty hours after infection, appeared somewhat shrunken from the loss of the secretions of the cephalic glands, but showed no increase in length over the free cercaria. One of these larvae was very remarkable in having the tail still attached to the body.

My smallest specimen from the livers of the mice was 0.22 mm. in length and 0.1 mm. in width. This specimen was recovered 12 days after infection. Specimens almost as small were recovered on the 10th day, the 14th day and the 18th day after infection (see Table I). It can be concluded from these results that there is but little increase in size until the worms reach the liver, since such specimens as have been found in passage to this organ are very small and the smallest forms found in the liver are but little larger than the cercariae.

Further, the finding of very small specimens in the liver, as late as 18 days after infection, might be explained by considering that such a form had been delayed in its passage to the liver, and that favorable conditions for growth in size are not found until this organ is reached.

During the second period there is by far the most rapid increase in size. As in other trematodes, *S. japonicum* reaches sexual maturity long before the maximum size is reached. My earliest record of copulation was nineteen days after infection. Individuals in copulation might be considered sexually mature, altho in the youngest pairs the uterus of the female did not yet contain eggs. The male in the youngest copulating pair which I measured, twenty-one days after infection, [Fig. 10 (F.) and 17 (M.)] had a length of 5.6 mm. and the female of 4.2 mm. It is interesting to note that in this early stage of copulation the female is considerably shorter than the male, while later the size relation is reversed. From these figures it can be seen that in the period of development in the liver of the mouse, up to sexual maturity, there can be an increase in less than twenty days from about 0.2 mm. to 0.3 mm. up to 5.6 mm. in the male, and 4.2 in the female, or an increase in length of 28 times and 21 times respectively. A comparison of the largest individual found on each of the days on which examinations were made indicates approximately the rate of growth during this period (see Table I). The largest individual, twelve days after infection, was 0.79 mm. in length, which is about five times the length of the cercaria, and about three

TABLE I.

Range of size in specimens found on different days.

	Number of days after first exposure to infection.						
	12	14	16	18	19	21	30
Male—							
Number of specimens measured*	10	8	9	7	13	13	10
Smallest	0.22	0.54	0.28	0.22	0.40	0.50	2.21
Largest	0.79	0.97	2.02	2.34	3.8	5.3	8.8
Female—							
Number of specimens measured*	12	15	10	13	10	10	10
Smallest	0.22	0.27	0.50	0.63	0.53	0.64	3.87
Largest	0.77	0.99	1.94	1.89	2.65	4.2	8.27

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* This does not represent the total number of specimens examined, since only those which were apparently near the upper or lower range were measured for this table.

times the length of the youngest form which I have found in the liver. From the twelfth to the nineteenth or twenty-first days, when copulation takes place, the increase in size is still more rapid, In these seven to nine days the largest individuals show a gain in length of from five to seven times. It is during this period that the increase in size is most striking, since the worms which are barely visible to the naked eye in the earlier stages, on the later days are easily seen and picked out. In fact, the growth was so rapid that it could be noted with the naked eye at the two day periods. It is also during this period that in my heavily infected mice the worms clogged the blood vessels of the liver, and seriously affected their hosts.

The third period of growth of *S. japonicum* in its final host extends from sexual maturity until maximum size is attained. During this period, the growth is slow and extends over a long period of time. We have no very exact information in regard to the actual rate of growth during this period or how long growth may continue. In my experimental mice, from the 21st to the 30th day, the growth in size was much less than in the corresponding time in the second period (Table I). My experiments give me no further data in regard to growth during this period. Katsurada (1914) records specimens in mice which reached the size of 8 mm. in the male and 11 mm. in the female, 62 days after infection.

Further data on growth during the final period of development may be obtained from Tables II and III which I have copied from Katsurada (1914, 365 and 366). The data included in these two tables give some idea of the rate of growth during the third period, and the length of time that growth continues.

The records of the specimens taken from the dogs 82 days, 10 months and 21 months after infection, are especially to the point. Full size seems to have been attained in 21 months, since the largest specimen at this time is as large as the largest individuals ever recorded.

Katsurada (1914, 365) comes to the following conclusion in regard to the size-relation in *S. japonicum*.

> Die Grösse der Tiere hängt von mancherlei Umständen ab, z. B. von ihren Entwickelungsstadien, von ihrer Individualität, von der Art des Wirtes und von sonstigen Verhältnissen. Nach unserer Beobachtung kann ein kleines Tierchen, dessen Körperlänge noch nicht ganz 5 mm. beträgt, schon reif sein.

His contention that the size of the host influences profoundly the size of the parasite is very interesting.

TABLE II.

Tabelle über Körperlänge des Schistosomum japonicum.

Autoren	Katsurada (1904).		Tsuchiya (1906).		Fuyinami (1907).		Katsurada (1909).		Katsurada (1911).		Katsurada (1913).	
Geschlecht	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Durchschnittl. Körperlänge (mm.)	10.43		16.1	21.8	16.0	17.8	10.92	14.5	8.0	11.0	12.8	10.6
Grösster Längendurchmesser (mm.)	12.0	12.0	19.5	26.0	21.0	23.0	13.0	17.0			15.0	12.0
Kleinster Längendurchmesser (mm.)	7.0	8.0	9.5	16.0	8.0	12.0	9.0	11.0			11.0	8.5
Zahl der Würmer	8		19	8			13	4			5	5
Zustand der Würmer	Formalinfixation		Frisch (aber 24 Std. n. d. Tod des Wirtes)				Formalinfixation		Frisch		Spiritusfixation	
Wirt	Katze		Mensch		Rind		Hund, gestorben, 82 Tage n. d. Ansteckungsversuch		Maus, getötet, 62 Tage n. d. Ansteckungsversuch		Hund, getötet, 10 Monate n. d. Ansteckungsversuch	

TABLE III.

Besonders gut entwickelte Schistosoma (sic) japonicum. Beispiel von 21 Monate alten Würmern—Wirt: männlicher Hund (Körpergewicht ungefähr 11 kg.)—frischer Zustand.

Geschlecht.		No.	Längendurchmesser (mm.).	Breitendurchmesser (mm.).		Mundsaugnapfdurchmesser (mm.). Querdurchmesser.	Bauchsaugnapfdurchmesser (mm.).	
							Querdurchmesser.	Längendurchmesser.
Unpaarige Schistosomen	♂	1 dickster	21.0	1.5		0.604	0.604	0.604
		2	20.0	1.2		0.513	0.581	0.467
		3	22.5	1.0		0.513	0.627	0.593
		4 dünnster	12.0	0.7		0.513	0.570	0.534
				Vorderkörper	Hinterkörper			
	♀	1 Längster	26.0	0.125	—	—	—	0.114
		2 Kürzester	18.0	0.137	0.445	—	—	0.091
Paarige Schistosomen	I Paar	♂	19.5	0.627	0.8	0.456	0.536	0.570
		♀	23.0	0.103	0.422	—	—	0.114
	II Paar	♂	19.5	0.570	0.8	0.502	0.559	0.570
		♀	21.0	0.103	0.513	—	—	0.103
	III Paar	♂	19.0	0.513	0.8	0.456	0.522	0.513
		♀	23.0	0.137	0.456	—	—	0.091

An important point which requires further discussion is the range of variation of individuals recovered on any given day after infection (see Table I). While this variation is very clear as early as the 12th day, with a maximum size of 0.79 mm. and a minimum of 0.22 mm., it is most strikingly exhibited during the later periods. On the 16th to the 19th days numbers of young distomes were found below 0.72 mm. in length, while the maximum on the 19th day had run up to 3.8 mm. in the male and 2.65 mm. in the female. Especially interesting was the finding of a male individual on the 18th day only 0.22 mm. in length. This specimen was very much contracted and probably represents about the stage of Fig. 2 (M.). By the 21st day no very small individuals were found, altho the range of variation was still quite great. By the 30th day the smallest individual found in a large series of examinations was a male, 2.21 mm. in length.

Since the mice were infected twice, with a lapse of about 17 hours between exposures, a slight degree of variation could be attributed to this cause. This cause is, of course, entirely inadequate to explain the great variations noted in Table I on all the days on which examinations were made, and especially the finding of very small individuals as late as 18 days after exposure to infection. To adequately explain these variations two things must be postulated, i.e., first a variation in the time taken for the passage from the skin to the veins of the liver, and second, the necessity of any given individual reaching the liver before there can be much increase in size. From the description given above, of the course of migration of the larval blood flukes, it seems certain that there must be a considerable variation in the time which is taken by the parasites in reaching their destination. From the large numbers which were found in the livers of the mice by the 12th day it seems probable that most of the larvae complete their course by this time. The view that only in the blood vessels of the liver can the young schistosomes find conditions favorable for their growth, is supported by the fact that the worms which have been found outside the liver in experimental animals, had increased but little in size over the cercaria. It is my opinion, therefore, that the range in size of the individuals found in the livers of my experimental mice was due to differences in the time taken in reaching the liver, and that the small individuals found in the later periods after infection, represented forms which had been considerably delayed in their way to the liver. That there is a definite limit to the time which may be taken in making this course, is indicated by the fact that on the 30th day no small worms were found. Apparently all the worms, that were going to reach the liver had already arrived.

3. *Changes in shape.*

Probably the most remarkable of the changes in the development of *S. japonicum* in its final host are the changes in shape. In the cercaria (Text Fig. 1) unless in an extreme state of extension the length of the body is about three times its width. The length of the post-acetabular region is only about one-half that of the pre-acetabular, and the cross-section is round. The shape is but slightly different in my smallest specimen from the liver [Fig. 1 (F.) or (M.)], in which the length is about two and one half times the width. The length of the post-acetabular region is still less than the pre-acetabular and the cross-section is oval. Since the shape of the adult males and females [Figs. 11 (F.) and 18 (M.)] are so remarkably modified by the sexual dimorphism, the study of the changes by which these peculiarities are attained through a series of developmental stages is interesting and significant. The shape of the adults is so strikingly different from that of the cercaria that it is very remarkable that such changes could be accomplished in the short period of 18 or 19 days which it takes the developing fluke to assume the characteristic shape of the adult. These changes consist in a very great increase of the length in ratio to the width, an enormous increase of the post-acetabular region of the body, as compared with the pre-acetabular, and a gradual assumption of the secondary sexual characteristics which produce the sexual dimorphism of the adult. Since the changes in shape are very different in the male and the female, the sexes will be discussed separately.

The ratio of length to width increases very greatly in the female (Table IV) as can be seen by a comparison of figures 3 (F.), 5 (F.), 7 (F.), 8 (F.) and 10 (F.). In these forms, the ratios of length to width are as follows: 3 (F.) = 33:10; 5 (F.) = 20.3; 7 (F.) = 12:1; 8 (F.) = 18:1; 10 (F.) = 28:1.

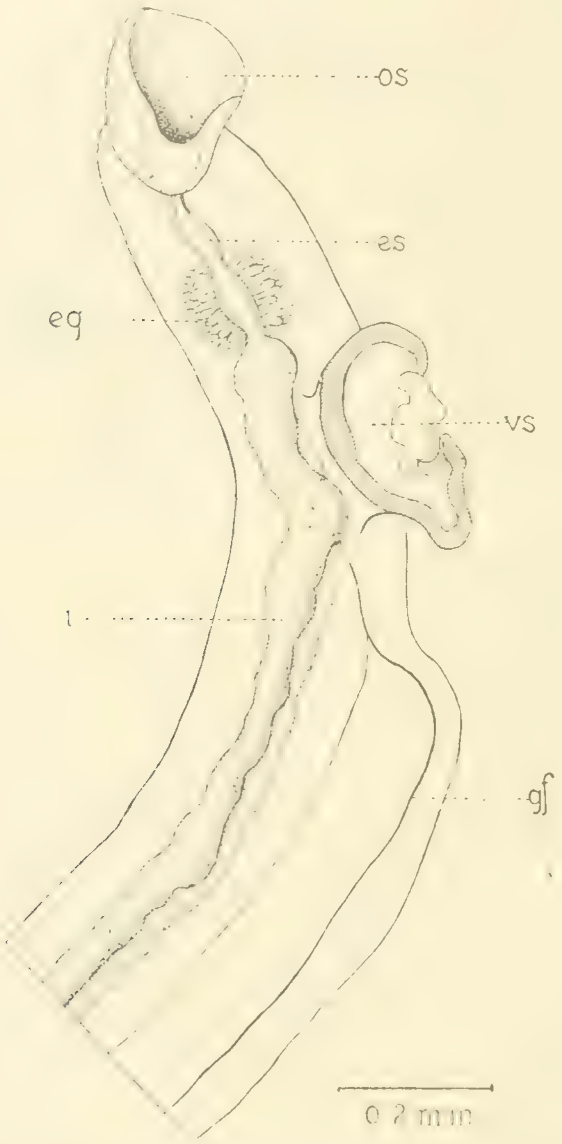
In the specimens, 30 days old, from my experimental mice this ratio varied from 50:1 to 90:1. The width in all these ratios was measured in the region of the ventral sucker. After the reproductive organs are fully developed, the post-ovarian region becomes considerably wider than the pre-ovarian. This is shown in Fig. 11 (F.), and Katsurada's measurements (see Table III) of three 21-month-old females, in copula, in which the ratio of length to width of the pre-ovarian region is about 200:1, while that in the post-ovarian region is only about 50:1.

The great increase in length of the female occurs almost entirely in the post-acetabular region and is correlated with the development of the reproductive organs and the extreme attenuation of the body. In my smallest form which can surely be considered a female [Fig. 2 F.)], the growth of the post-acetabular region has already brought the acetabulum in front of the middle of the body, and the pre-acetabular region is 48 per cent. of the total body length. In a series of females the percentage of the total body length occupied by the pre-acetabular region decreases rapidly with growth, as follows: Fig. 3 (F.) = 43 per cent.; Fig. 4 (F.) = 37.5 per cent.; Fig. 5 (F.) = 11 per cent.; Fig. 10 (F.) = 6 per cent. In the females of the thirty-day period this percentage has been reduced as low as 3 to 4 per cent. The pre-acetabular region also becomes distinctly narrower with growth.

Another change in the shape of the body of the female is an increase in thickness which changes the shape of the cross-section from an oval, in the early stages, to a circle in the later stages. This modification is completed at about the size of 7 (F.). When the body has become rounded in cross-section the young female worm comes to lie on the side and tends to curl up [See Figs. 8 (F.) and 9 (F.)].

In the males of *S. japonicum* the changes in shape are of an entirely different character from those in the female. In the male the length increases very much in ratio to the width, but not to the extent noted above, for the female. The changes in the ratio of the length to the width up to the time of the formation of the gynaecophoric canal are illustrated by the following series, i.e., Fig. 2 (M.) = 30:11; Fig. 4 (M.) = 44:13; Fig. 7 (M.) = 5:1; Fig. 9 (M.) = 6:1. After the formation of the gynaecophoric canal this change in ratio is even more rapid, as is shown by the following series: Fig. 11 (M.) = 7:1; Fig. 14 (M.) = 9:1; Fig. 17 (M.) = 16:1.

Also the increase in length of the post-acetabular region is not quite as great, as in the female. In a typical male series, the percentage of body length which is occupied by the pre-acetabular region, is as follows: Fig. 5 (M.) = 40 per cent.; Fig. 7 (M.) = 31 per cent.; Fig. 10 (M.) = 22 per cent.; Fig. 14 (M) = 21 per cent. As development proceeds, the pre-acetabular region becomes distinctly set off from the rest of the body (Fig. 10 (M.) and Text fig. 3), and becomes round in cross section, not sharing in the flattening of the post-acetabular region. in connection with the formation of the gynaecophoric canal.



Text fig. 3. Anterior region of half-grown male specimen of *S. japonicum*, to show an early stage in the development of the gynaecophoric canal; *eg*, esophageal gland; *es*, esophagus; *gf*, fold of gynaecophoric canal; *i*, intestine; *os*, oral sucker; *vs*, ventral sucker.

As noted above, from an early stage in development, the post-acetabular region of the male is distinctly wider than in the female. Also, the body in this region tends to become thinner [Fig. 4 (M.), 5 (M.), 10 (M.)]. In the earlier stages the post-acetabular region is not set off from the rest of the body. It is only when the length of the young distome approaches 2 mm. that the characteristic shape of the post-acetabular region begins to be assumed. At this stage the post-acetabular region is set off distinctly from the pre-acetabular, the widening coming just behind the base of the acetabulum, which at this stage juts out considerably from the body.

Just after this stage, the sides of the thin post-acetabular region begins to grow up to form a trough [Figs. 11 (M.), 13 (M.), 14 (M.) and Text fig. 3]. As soon as this growth comes about, the shape of the male becomes such that it tends to turn over on its side and often to curve toward the dorsal side [Fig. 13 (M.)]. Soon the folding over becomes so great that the opening of the groove is almost closed by the sides of the body [Fig. 14 (M.)]. At this stage, the canal is almost completely formed, and is soon ready to enclose the female. During the course of this development, the body has become very thin.

4. *Development of the ventral sucker or acetabulum.*

In my description of the cercaria of *S. japonicum*, the following statement is made in regard to the ventral sucker (Cort, 1919, 494). "The ventral sucker is located at a point about three fourths of the distance from the anterior to the posterior end of the body. It is circular in ventral view with a depth considerably greater than its diameter. In the living animal the ventral sucker can be protruded so that it has almost the appearance of a proboscis. The sucker functions strongly in locomotion and is able to hold the cercaria against considerable pressure." As shown in Text fig. 1 the ventral sucker of the cercaria has only about one fourth the length of the oral sucker and one third its width. It has a diameter varying from about 0.018 mm. to 0.022 mm. Contrast with this the large size of the ventral sucker of the adults, especially the males, or even of the copulating pairs [Fig. 10 (F.) and 17 (M.)]. Katsurada (1914, 366) gives measurements of the ventral suckers of specimens taken from a dog 21 months after infection (See Table III). In this material the diameter of the ventral sucker varies, in the male from 0.5 mm. to almost 0.63 mm., and in the female from 0.091 to 0.114. This difference in the size of the ventral sucker of the male and female is apparent at a very early stage, and is one of the ways in which the sexes can be distinguished in the young worms [cf. Figs. 4 (F.) and 4 (M.)]. The stages in the development of the ventral sucker can be followed step by step in the females, and the increases in size at the various stages seen in Table IV. The change in position of the ventral sucker, due to the relatively great increase in length of the post-acetabular region has been discussed in another connection.

In my youngest form [Fig. 1 (M. or F.)], in which I am not sure of the sex, the ventral sucker has almost doubled in size over the cercaria, having a length of 0.034 mm. and a width of 0.038 mm. In Fig. 4 (F.) in which the length of the body is almost twice that of Fig. 1 (F. or M.) the ventral sucker has a length of 0.067 mm. and a width of 0.068 mm. Beyond this stage, the increase in size of the ventral sucker of the female is more gradual, and not in proportion to the increase in body length. This can be seen by comparing Fig. 4 (F.) with Fig. 8 (F.), in which the length of the body has been almost quadrupled with only a 50 per cent. increase in the size of the ventral sucker (See Table IV). Beyond this stage, there is very little further increase in the size of the ventral sucker, although the length of the body increases very greatly. This is shown by a comparison of 8 (F.), with a total length of 1.8 mm., and a ventral sucker with a diameter of 0.099 mm., and the female from Katsurada's first copulating pair (see Table III), in which the length of the body was 23 mm., and the diameter of the ventral sucker only 0.114 mm.

In the early stages of the development of *S. japonicum* in its final host the ventral sucker is like that of any young distome. As the female elongates, so that the ventral sucker comes to lie in the anterior third of the body, it becomes thinner and begins to be distinctly set out from the surface of the body [Figs. 6 (F.)]. At the stage shown in Fig 7 (F.) the ventral sucker is saucer-shaped and somewhat pedunculate, and by the time the female becomes circular in cross-section, it assumes the characteristic adult shape of a shallow saucer set on a short stalk [Fig. 8 (F.)].

In the male the great increase in the size of the ventral sucker can be seen from my series of figures [Figs. 2 (M.) to 17 (M.)], and by comparing the measurements in Table IV. Here, as in the female, the rate of increase in size is greater in the earlier stage of development, but continues until growth is completed. Therefore, in comparing Fig. 3 (M.) with Fig. 4 (M.) (See also Table IV) it will be noted that while the increase in length is only about 50 per cent., the size of the ventral sucker has been doubled, while in the growth from the stage of Fig. 4 (M.) to that of Fig. 7 (M.) the length has been almost doubled, while the increase in size of the ventral sucker has been less than 50 per cent. A still further slowing of the rate of growth is apparent from a comparison of Fig. 7 (M.) with Fig. 14 (M.) in which the length has been almost quadrupled, while the size of the ventral suckers has been only a little more than doubled (see also Table IV). This rate of increase becomes still less in the later stages. This can be clearly shown by a comparison of Fig. 14 (M.) with the adult male [Fig. 18 (M.)].

In the male the change in shape of the ventral sucker, during development, is even greater than in the female. At early stages in the male, the ventral sucker becomes very thin and flexible, as is shown by the irregularity of its shape, and it juts out from the body [Figs. 4 (M.) and 5 (M.)]. At an early stage in the formation of the gynaecophoric canal the ventral sucker has assumed the characteristic shape of the adult [Fig. 11 (M.) and Fig. 14 (M.)]. The ventral sucker is very thin and flexible, with the outline quite irregular and varying from saucer shape to cup shape. It is always set off from the body with a distinct stalk, and after the formation of the gynaecophoric canal it is set in the hollow formed between the rounded neck region and the anterior limits of the sides of the gynaecophoric canal (Text fig. 3).

There is, of course, a considerable degree of variation in the size of the ventral sucker in individuals of the same length. This variation, however, is not sufficient to invalidate the course of development as outlined above.

3. *Development of the oral sucker.*

The oral sucker of the cercaria of *S. japonicum* has been described in an earlier paper (Cort, 1919, 496 to 497), and is shown in Text figs. 1 and 2. The oral sucker at this stage has scarcely any of the permanent characteristics of the adult, and its structure is very much modified by the presence of adaptive larval characters, and by its relation to the activities of the cercaria in locomotion and penetration. The oral sucker of the cercaria occupies almost one-third of the body length, and the mouth and buccal cavity are very small (Text figs. 1 and 2, M.). The oral sucker of the adult [Figs. 11 (F.) and 18 (M.)] is entirely different from that of the cercaria. While it has increased very greatly in size, it has come to occupy only a very small fraction of the body length. The oral sucker has now assumed the shape of a funnel with a very large mouth and thin lips. The buccal cavity is funnel-shaped and small at the point where it joins the esophagus. The mouth opens on the ventral side, and the shape of the sucker and buccal cavity is asymmetrical. At the stages shown in Fig. 8 (F.) and Text fig. 3 the oral sucker has already assumed its characteristic adult condition. A comparison of these figures with the cercaria show how profoundly this structure has been modified in development.

In the smallest individual which I have figured [Fig. 1 (F. or M.)] the oral sucker has already changed fundamentally from the condition of the cercaria. The adaptive larval characters, i.e., the anterior spines, the ducts of the cephalic glands and the "head gland" have already disappeared. The mouth and buccal cavity have increased in size, the latter being already funnel-shaped. The characteristic ventral position of the mouth is apparent at this stage, establishing definitely its relation to the mouth, as shown in the cercaria [Text fig. 1 (M.)]. While the diameter of the mouth at this stage is greatly increased over the condition of the cercaria, it is as yet only a little over one fourth the diameter of the oral sucker. The oral sucker in this specimen occupies slightly over one fourth of the body length, the length of the body in this specimen being 0.24 mm., and the length of the oral sucker 0.063 mm. The oral sucker at even this early stage is much more definitely set off from the rest of the body than in the cercaria, and its muscles are developed in relation to the sucking activity of the mouth and buccal cavity.

Early in the development there is a greater proportional increase in size in the oral sucker of the male than of the female. In fact, the easiest way to distinguish the sexes in the earlier stages is by a comparison of the size of the oral suckers. This difference is shown clearly by comparing the increases in size of the oral sucker in relation to the increases in length of the body of a series of females [Figs. 2 (F.), 3 (F.), 4 (F.), 5 (F.), 7 (F.) and 8 (F.) [, with a comparable series of developmental stages of males [Figs. 3 (M.), 4 (M.), 7 (M.), 9 (M.), 10 (M.), 14 (M.)] (see also Table IV). In this series of females the body length increases from 0.27 mm. to 1.85 mm., while the diameter of the oral sucker only increases from 0.05 to 0.092 mm. On the other hand, in the series of males the body length increases from 0.3 mm. to 3.1 mm., and the diameter of the oral sucker from 0.074 mm. to 0.227 mm. Katsurada records in fully developed males (Table III) measurements of the oral sucker ranging from 0.456 mm. to 0.604 mm. There is very little, if any, increase in size in the oral sucker of the female beyond the stage of 8 F.

The ratio in size of the oral sucker and the ventral sucker shows an interesting fluctuation in the course of the development, both of the male and the female. In the cercaria the oral sucker is between three and four times the size of the ventral sucker. At the stage in development, represented by Figs. 4 (F.) and 4 (M.) the suckers are very nearly equal in size. From this time on (see Table IV), the ratio is reversed, and the ventral sucker is constantly larger than the oral sucker.

The most striking change in the development of the oral sucker is the increase in the size of the mouth and buccal cavity. Even at the stage of Fig. 1 (F. or M.) the width of the mouth has already increased greatly beyond the condition in the cercaria (Text figs. 1 and 2), and the buccal cavity is beginning to assume the characteristic funnel shape. As development proceeds, the mouth increases rapidly in size [cf. Figs. 2 (M.), 3 (M.), 4 (M.), or Figs. 2 (F.), 3 (F.), 4 (F.)], coming to occupy most of the ventral surface of the oral sucker. The buccal cavity soon assumes the condition approximating the adult [Text fig. 3 and Fig. 8 (F.)]. It can be seen, by comparing the oral suckers at the stages of these figures with the condition in the cercaria (Text fig. 2), that the asymmetrical condition of the oral sucker which is apparent in the cercaria persists in later stages. In Text fig. 3, for example, it can be seen that the distance from the anterior lip of the oral sucker to the passage into the esophagus is longer than the distance from the posterior lip to this point, and that consequently the same relation is true in the buccal cavity.

6. *Changes in the digestive system.*

The digestive system in the cercaria of *S. japonicum* (Text figs. 1 and 2) is not functional, and is in a very rudimentary stage of development. This is especially indicated by the fact that the intestinal region, which attains such an extensive development in the adult [Figs. 11 (F.) and 18 (M.)], is represented in the cercaria only by a heart-shaped sac (Text fig. 1, *ds*). The remarkable changes which this system must pass through to attain the adult condition may be divided into three periods, i.e., (1) from the condition in the cercaria to the beginning of functional activity, (2) from the beginning of functional activity to the union of the intestinal ceca, (3) from the union of the ceca until completion of development. Of the first period, we know but little, altho Miyagawa's (1916) account of the development of this system indicates that functional activity is attained very soon after penetration (see literature section). The second period is characterized by the growth of the intestinal ceca. During this period, as indicated by Miyagawa's (1916) account and my own earlier stages, there is intense functional activity which is indicated by the very great distension of the intestine with food material [Figs. 3 (F.) and 3 (M.)]. The third period is characterized by a very rapid extension of the intestine, to keep pace with the growth of the body.

My earliest stage [Fig. 1 (M. or F.)] shows a great advance in the development of the digestive system over the cercaria, since the intestinal ceca are well developed and distended with brownish food material, indicating functional activity. There is, therefore, a big gap in my material, which includes the early stages of organization of the digestive system in preparation for functioning. Miyagawa's account (see section on literature) partly fills this gap. This organization must come very early, since the young worm needs an abundant supply of food, as soon as it has entered the final host, to prepare itself for the period of rapid growth. In my earliest stage [Fig. 1 (M. or F.)] the esophagus is well developed and already surrounded by the characteristic glandular area. This esophageal gland is made up of closely packed unicellular glands, which open separately into the esophagus. The only noticeable changes in the esophagus, as development proceeds, are increases in its diameter and length, corresponding to increases in size of the pre-acetabular region.

The development of the intestinal ceca up to the time of union is shown in Figs. 1 (M. or F.), 2 (M.), 2 (F.) and 3 (M.). These figures show an elongation of the intestinal ceca, corresponding to the increase of the post-acetabular region, and an approach of the ends of the ceca which fore-shadows their union. The most characteristic thing during this period is the great distension of the intestine with brownish food material, which makes its bulk very large in proportion to the size of the post-acetabular region of the body. The union of the intestinal ceca comes, when the developing worms have reached a length of about 0.3 mm. to 0.4 mm. [Figs. 3 (F.) and 4 (M.)]. For a considerable time after the union of the ceca there is still noticeable a considerable distension of the intestine with food material [Figs. 5 (F.), 5 (M.) and 7 (M.)]. As the length of the post-acetabular region of the body increases, and the intestine becomes longer, it is less distended with food material, and assumes more the narrow irregular appearance of the fully developed condition [Figs. 5 (F.), 7 (F.), 8 (M.) and 9 (M.)]. In the later stages of development the intestine of the male becomes wider than that of the female. From cross-sections, however, it can be seen that this difference is compensated for, in the female, by a greater thickness of the intestine. These differences are of course, correlated with the differences in thickness of the body in the two sexes.

The relative length of the divided and the united regions of the intestine, at various stages, can be seen from Table IV. At the time of union of the intestinal ceca, two points are definitely established, i.e., (1) the point of bifurcation of the intestinal ceca which remains throughout all the further stages of development just in front of the base of the ventral sucker, and (2) the posterior tip of the intestine which remains always very close to the posterior tip of the body. As the body length increases, these two points remain constant and there is an increase in length of both the divided and the united portion of the intestine, to keep pace with the increase in the body length.

Soon after the union of the intestinal ceca a distinct sexual difference can be noted. In the adult female, the area just in front of the intestinal ceca is much wider than in the male, since it contains the ovary and other parts of the reproductive system. An examination of the figures of the early stages of the female [Figs. 3 (F.), 4 (F.), 5 (F.), 6 (F.)] shows that this area is already apparent at an early stage of development, and contrasts with the condition of this region in the male [Figs. 4 (M.), 7 (M.), 8 (M.)]. This difference is one of the points of sexual dimorphism which makes early discrimination of the sexes possible.

7. *Development of the reproductive organs.*

No attempt was made to trace the histogenesis of the reproductive organs. I will note, however, certain of the important stages in development. It is only late in development that the reproductive organs themselves become clearly differentiated. In the male it was difficult to make out the outlines of the reproductive organs in the early stages. In a male, 2.5 mm. in length, taken from a mouse nineteen days after infection, the outlines of the seminal vesicle and the testes were clear. The testes were almost round, and composed of dense nuclei, the spermatogonia, with considerable space between. The testes at this stage had an embryonic appearance, and there was no trace of spermatogenesis. The length of the total testes group of seven, was 0.175 mm., and the testes had a diameter ranging from 0.023 to 0.032 mm. The testes had distinct spaces between them. A

TABLE IV.

Measurements of a series of development stages of Schistosoma japonicum in mm.[*]

Individual Characteristics.	1 (F. or M.).	2 (F.).	3 (F.).	4 (F.).	5 (F.).	6 (F.).	7 (F.).	8 (F.).	10 (F.).	11 (F.).	2 (M.).	3 (M.).
Length	0.24	0.27	0.325	0.48	0.81	0.91	1.1	1.85	4.2	8.2	0.3	0.3
Width at ventral sucker	0.1	0.1	0.1	0.125	0.12	0.11	0.1	—	—	0.1	0.11	0.115
Width at middle of pre-acetabular region	0.08	0.085	0.085	0.075	0.1	0.09	0.075	—	—	—	0.095	0.1
Width at middle of post-acetabular region	0.1	0.1	0.115	0.12	0.14	0.12	0.12	0.1	0.15	—	0.095	0.12
Length of pre-acetabular region	0.15	0.13	0.14	0.18	0.23	0.2	0.12	0.2	0.25	0.45	0.16	0.15
Percentage of total length taken up by pre-acetabular region	62.0	48.0	43.0	37.5	28.0	22.0	10.0	11.0	6.0	5.0	53.0	50.0
Ratio of length to width	—	—	33 : 10	—	20 : 3	—	12 : 1	18 : 1	28 : 1	—	30 : 11	—
Length of oral sucker	0.063	0.059	0.069	0.067	0.071	—	0.057	0.075	—	0.1	—	0.069
Width of oral sucker	0.055	0.05	0.05	0.063	0.084	—	0.092	—	—	—	—	0.074
Length of ventral sucker	0.034	0.042	0.053	0.067	0.067	—	0.067	0.099	—	0.1	—	0.035
Width of ventral sucker	0.038	0.059	0.061	0.068	0.084	—	0.097	—	—	—	—	0.059
Length of bifurcated part of intestine	0.101	0.126	0.153	0.168	0.378	—	0.609	—	—	—	—	0.161
Length of united part of intestine	—	—	0.042	0.095	0.189	—	0.174	—	—	—	—	—
Width of intestine	0.025	0.025	0.044	0.067	0.042	—	0.021	—	—	—	—	0.032

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[*] The numbers given to the individuals in Table IV correspond to the numbers given to the figures in Plates I. to IV.

TABLE IV (*continued*).

Individual Characteristics.	4 (M.).	5 (M.).	7 (M.).	8 (M.).	9 (M.).	10 (M.).	11 (M.).	13 (M.).	14 (M.).	15 (M.).	17 (M.).	18 (M.).
Length	0.44	0.56	0.8	1.07	1.5	1.8	1.7	2.2	3.1	3.8	5.6	8.1
Width at ventral sucker	0.13	0.15	0.16	0.21	0.25	0.24	0.25	—	—	—	—	0.2
Width at middle of pre-acetabular region	0.11	0.12	0.12	0.16	0.22	0.18	—	—	—	—	—	—
Width at middle of post-acetabular region	0.14	0.15	0.14	0.19	0.22	0.28	—	—	—	—	—	—
Length of pre-acetabular region	0.21	0.26	0.25	0.34	0.47	0.4	0.5	0.5	0.65	0.6	0.85	0.7
Percentage of total length taken up by pre-acetabular region	47.0	40.0	31.0	32.0	31.0	22.0	29.0	23.0	21.0	16.0	18.0	9.0
Ratio of length to width	44 : 13	—	5 : 1	5 : 1	6 : 1	—	—	—	9 : 1	—	16 : 1	—
Length of oral sucker	0.088	—	0.105	—	0.202	0.168	0.197	—	0.179	0.252	—	0.3
Width of oral sucker	0.103	—	0.128	—	0.17	0.176	—	—	0.227	—	—	—
Length of ventral sucker	0.082	—	0.122	—	0.208	0.242	0.210	—	0.273	0.302	—	0.3
Width of ventral sucker	0.095	—	0.128	—	0.21	0.252	—	—	0.294	—	—	—
Length of bifurcated part of intestine	0.179	—	0.357	—	0.777	1.144	0.79	—	1.59	1.69	—	—
Length of united part of intestine	0.08	—	0.168	—	0.225	0.426	0.336	—	0.84	1.4	—	—
Width of intestine	0.042	—	0.057	—	0.021	0.063	—	—	—	—	—	—

more advanced stage in development was seen in a male, 3.7 mm. in length, taken from a mouse twenty-one days after infection. The testes in this form were denser and appeared to be in the process of spermatogenesis. This male was just a little smaller than the smallest male found in copulation. Probably, therefore, it represents the stage of the beginning of sexual maturity. The total length of the testes group in this form was 0.205 mm. The individual testes were compact bodies, somewhat rounded, but wider than long. The length of the individual testes was about 0.023 to 0.032 mm., and their width 0.042 to 0.048 mm. The anterior and posterior testes were almost round, and distinct spaces were visible between the individual testes.

The fully matured condition was represented by a male 8 mm. in length [Fig. 18 (M.)], taken from a mouse thirty days after infection. In this specimen the total length of the testes area was 0.532 mm. The dorso-ventral diameter of the middle five testes was greater than their length, while the anterior and posterior ones were practically spherical. The smallest testes in the group had a width of 0.095 mm. and a length of 0.067 mm., while the largest had a length of 0.076 mm. and a width of 0.105 mm. The diameter of the end ones was 0.095 mm. These testes were very compact, surrounded by a definite connective tissue sheath, and were in contact.

In males of about 1 to 2 mm., traces of the reproductive organs could be made out, but their outlines could not be clearly distinguished. In earlier stages I was unable, in the material studied, to find the *Anlage* of the male system.

In very early stages the *Anlage* of the ovary can be distinguished in the widened area in front of the place of union of the intestinal ceca. The smallest form in which I was able to distinguish any of the other organs was a specimen 1.44 mm. in length, taken from the liver of a mouse, eighteen days after exposure to infection. At this stage the ovary had a length of 0.095 mm. and a width of 0.030 mm. Just in front of the ovary the position of Mehlis' gland could be distinguished. The uterus was very clearly defined and could be traced, as a straight tube, in the region between the intestinal ceca, from just in front of Mehlis' gland to the genital pore. Parts of both the vitelline duct and oviduct could be distinguished, and the position of the vitellaria was indicated by denser areas in the posterior end of the body. At this early stage, therefore, all parts of the reproductive system are differentiated.

In another specimen, 3.2 mm. in length, which was much coiled, the ovary had a length of 0.171 mm. and a width of 0.042 mm. The outlines of the lobes of the vitellaria could be clearly distinguished along each side of the body for the whole length of the post-ovarian region, but they did not have the characteristic brownish color which comes with functional activity. The outlines of the other organs were clearly defined. This form evidently represents a stage, just before sexual maturity was attained. In a fully matured specimen [Fig. 11 (F.)] the ovary had a length of 0.4 mm. and a width of 0.15 mm., the vitellaria were very dense, and had a brownish color. There were in this specimen about 50 eggs in the uterus.

VII. Summary.

1. The contrast is very striking between the cercaria of *S. japonicum,* in which the structure of the body is greatly modified by adaptive larval characters for locomotion and penetration, and the adult in which the sexes are separate and there is an extreme sexual dimorphism.

2. Sexual dimorphism has never been demonstrated in the cercariae of the human schistosomes, although the finding of a size dimorphism in a closely related species of schistosome cercaria suggests that undetected differences may be present.

3. The finding by Japanese workers, that when an experimental host is infected with cercariae of *S. japonicum* from a single snail, all the resulting individuals are in the great majority of cases of the same sex, and my own findings that in the schistosome cercaria from Douglas Lake, in fifteen infected snails studied, all the cercariae from a single snail were of the same size type, suggests the theory that sex in the schistosomes is differentiated in the miracidium stage, and that all cercariae which develop from a single miracidium are of the same sex.

4. The work of Japanese investigators on the life history of *S. japonicum* has demonstrated that infection with this species is always by penetration of the cercaria through the skin or the oral or pharyngeal mucous membrane, and never by way of the digestive tract, and that the cercariae can penetrate easily at any point through wet skin, although the most frequently enter by way of the hair follicles.

5. The course by which the larval blood fluke reaches the blood vessels of the liver has been the subject of considerable controversy. Soon after exposure to infection, the larvae will be found in the epidermis, dermis and subcuticula, and later most of them enter the

small blood vessels and lymph spaces. They are carried to the heart, either by the venous system or by the lymph vessels, although the former seems to be the most frequent course, since most of those which get into the lymph system seem to die in the lymph glands. From the heart they are carried to the lungs. From the blood vessels of the lungs, two courses have been traced. The first course is through the capillaries of the lungs back to the heart, from the heart by the arterial system, and through the capillaries to the hepatic portal system. Those larvae which follow the second course which is probably by far the most frequent, leave the blood vessels in the lungs, penetrate through the lung tissue, enter the peritoneal cavity by way of the connective tissue of the posterior mediastium and to a lesser extent of the anterior mediastinum, following with preference the course of the large blood vessels and nerve trunks and finally penetrate into the liver, where they enter the hepatic portal system.

6. Soon after penetration throuh the skin, the tail of the cercaria is lost and the cephalic and head glands degenerate. There is very little increase in size, until the larvae reach the liver, altho the digestive system becomes organized for functioning, and the oral sucker begins to assume its true character.

7. In the earliest stage found in the liver, it is impossible to distinguish the sexes, but at the stage between 0.3 mm. to 0.4 mm. the males show distinctly larger suckers, and a broader body than the females, and the females have a distinctly broader area in front of the union of the intestinal ceca, where the ovary later develops.

8. To attain the largest size recorded for this species the cercaria must increase in length over 100 times. The most rapid period of growth is from the time of reaching the liver to sexual maturity, when the worms may increase their length in less than 20 days by 20 to 30 times. Growth continues after sexual maturity, but is much slower.

9. The finding of larvae as small as 0.3 mm. in the liver, up to eighteen days after infection, can be explained by postulating that the worms grow but little in size, until they reach the liver, and that there is a variation in time for the migration course.

10. The increases in size are almost entirely in the post-acetabular region. As growth goes on, there is a very great increase in the ratio of length to width, which is much greater in the female than in the male. The body of the female soon becomes much narrower than in males of the same age, and becomes round in cross-section, while in the male the post-acetabular region gradually becomes flattened, and the sides grow up to form the gynaecophoric canal.

11. The size of the suckers increases greatly in the course of development, but not in proportion to the increases in body size. In the male, this increase is much greater than in the female. In the cercaria the ventral sucker is only about one third of the diameter of the oral sucker. In an early stage of development, the suckers become about equal in size, and in later stages the ventral sucker is constantly larger than the oral sucker. The ventral sucker with increase in size becomes thinner, and juts out from the body, until in later stages it is saucer-shaped and has a distinct peduncle. The greatest change in the oral sucker is the great increase in the size of the mouth and buccal cavity.

12. From the very rudimentary condition of the cercaria the digestive system soon becomes horseshoe shaped, and very much distended with food material. The ends of the intestinal ceca unite at a stage of about 0.4 mm. in length. The divided and united portions both extend greatly, to keep pace with the increased length of the body. In the female the position of the point of union of the intestinal ceca and the greater width of the area in front of this point as compared with the male is influenced by the development here of the ovary.

13. Differentiation of the reproductive organs comes late. Although at an early stage, the *Anlage* of the ovary can be distinguished, it is not until the female attains a length of about 1.5 mm., that the outlines of the ducts and other organs can be clearly made out. In the male it was only in specimens over 2 mm. in length that the outlines of the testes could be clearly distinguished.

EXPLANATION OF PLATES.

All figures were drawn with a camera lucida and represent different stages in the development of *S. japonicum,* in experimentally infected mice. The numbers of the figures correspond to the numbers given to the individuals in Table IV.

Abbreviations used:

e, eggs; *eg,* esophageal gland; *es,* esophagus; *gf,* fold of gynaecophoric canal; *i,* intestine; *is,* intestinal space in female, where ovary develops; *n,* central nerve mass; *os,* oral sucker; *ov,* ovary; tt, testes; *vit,* vitellaria; *vs,* ventral sucker.

PLATE I.

Fig. 1 (F. or M.) Very immature specimen from liver of mouse, ten days after infection; length 0.24 mm., sex not certain, ventral view.

Figs. 2 (F.), 3 (F.) and 4 (F.). Very immature females from mouse's liver, twelve days after infection, ventral view.

Figs. 2 (M.) and 3 (M.). Very immature males from liver of mouse, twelve days after infection, ventral view.

Fig. 4 (M.). Very immature male from liver of mouse, sixteen days after infection, ventral view.

Plate II.

Figs. 5 (M.) and 7 (M.). Immature males from liver of a mouse, fourteen days after infection, ventral view.

Figs. 8 (M) and 9 (M.). Immature males from the liver of a mouse, sixteen days after infection, ventral view.

Fig. 5 (F.). Immature female from liver of a mouse, fourteen days after infection, ventral view.

Fig. 6 (F.). Immature female from liver of a mouse, sixteen days after infection, ventral view.

Fig. 7 (F.) Immature female from liver of a mouse, eighteen days after infection, ventral view.

Fig. 8 (F.). Immature female from liver of a mouse, nineteen days after infection, side view.

Plate III.

Fig. 9 (F.). Immature female from liver of a mouse, nineteen days after infection, side view.

Fig. 10 (M.). Immature male from liver of a mouse, twenty-one days after infection, ventral view.

Fig. 11 (M.). Immature male from liver of a mouse, nineteen days after infection, side view.

Fig. 13 (M.). Immature male from liver of a mouse, eighteen days after infection, side view.

Fig. 14 (M.). Immature male from liver of a mouse, nineteen days after infection, ventral view.

Figs. 17 (M.) and 10 (F.). Male and female in copulation from liver of infected mouse, twenty-one days after infection.

Plate IV.

Fig. 18 (M.). Sexually mature male from liver of mouse, thirty days after infection.

Fig. 11 (F.). Sexually mature female from liver of mouse, thirty days after infection.

LITERATURE CITED.

Cort, W. W.

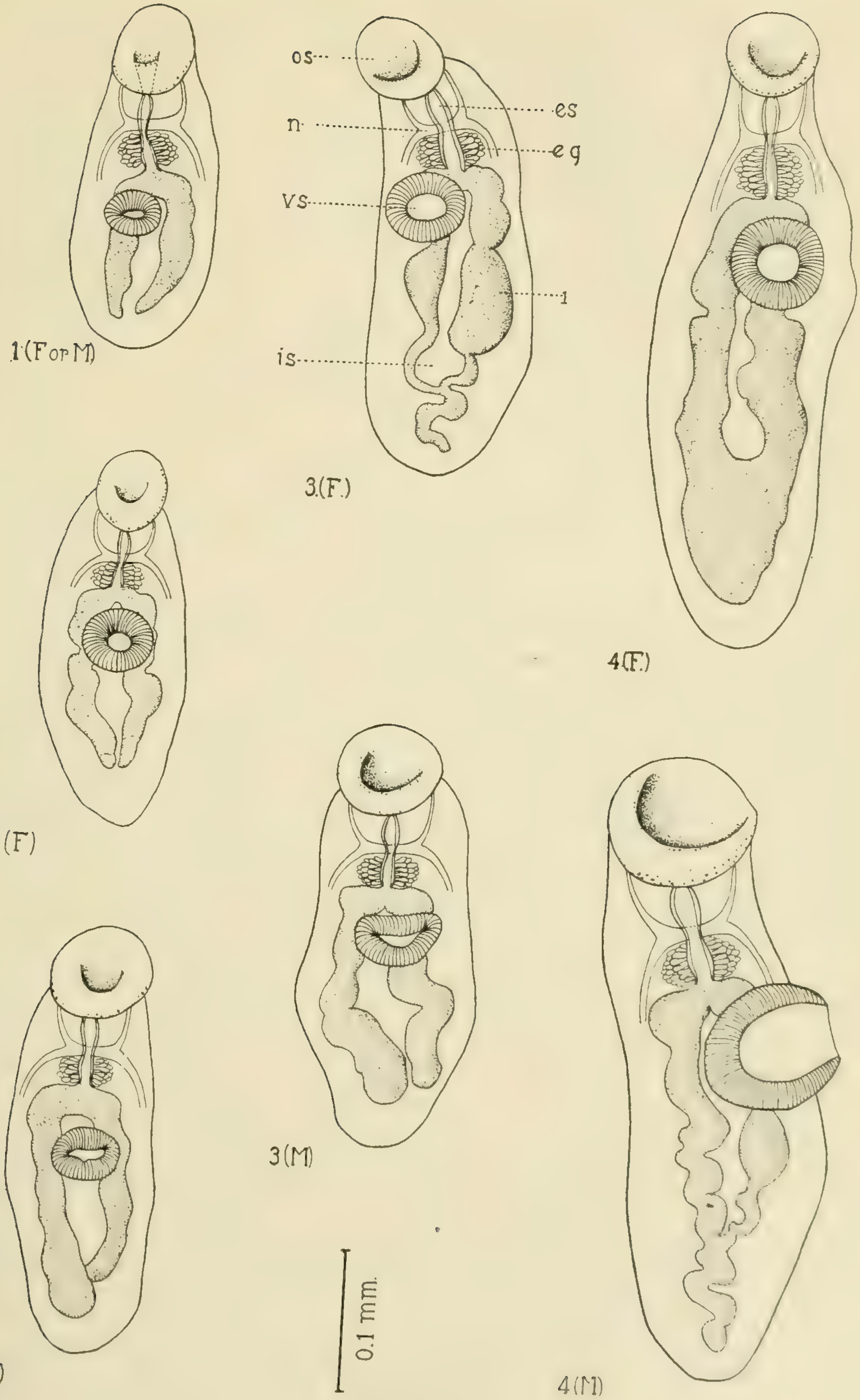
1919. The cercaria of the Japanese blood fluke, Schistosoma japonicum Katsurada. Univ. Calif. Publ. Zool., XVII, 485–507, 3 figs. in text.

Faust, E. C.

1919. Notes on South African cercariae. Jour. Parisit., V, 164–175, 1 pl.

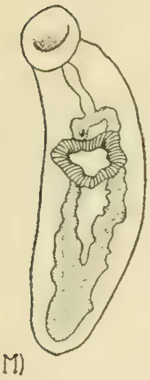
Fujinami, A.

1914. Ueber die Bekämpfung der japanischen Schistosomum Krankheit, nebst einer kurzen Schilderung der Pathologie dieser Krankheit.



1 (F or M)
3 (F.)
4 (F.)
2 (F)
3 (M)
2 (M)
4 (M)

PLATE I.



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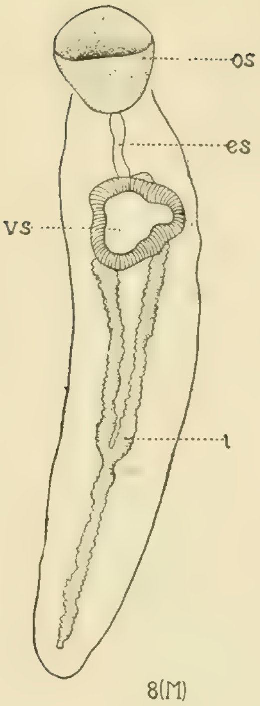

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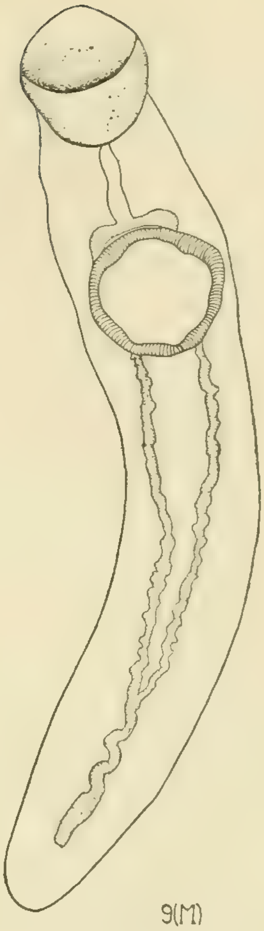

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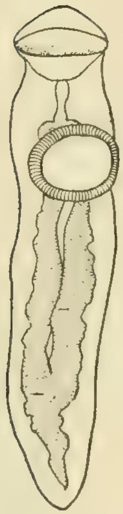

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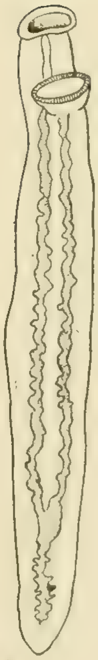

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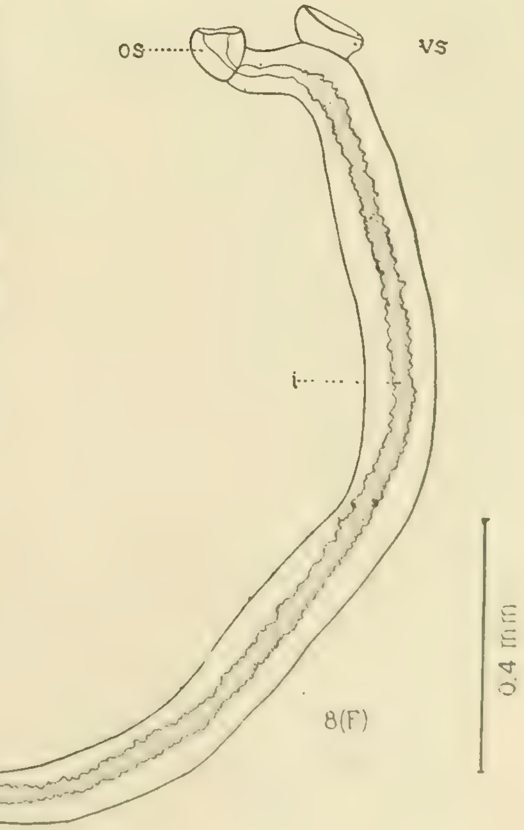

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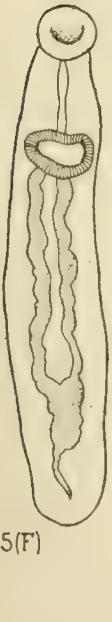

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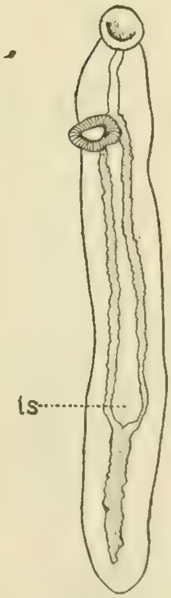

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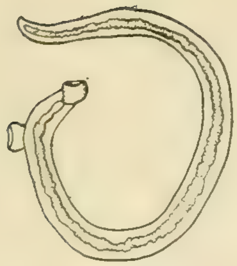
Plate II.



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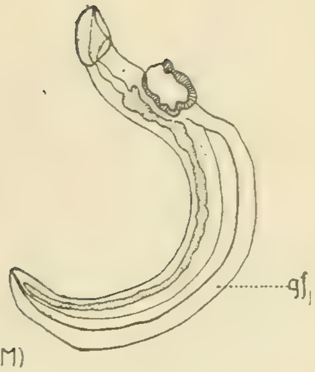

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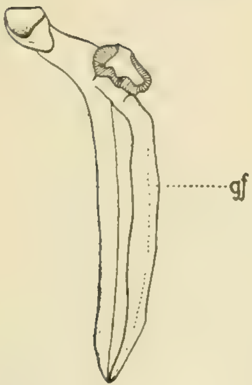

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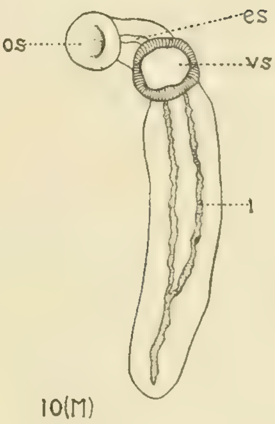

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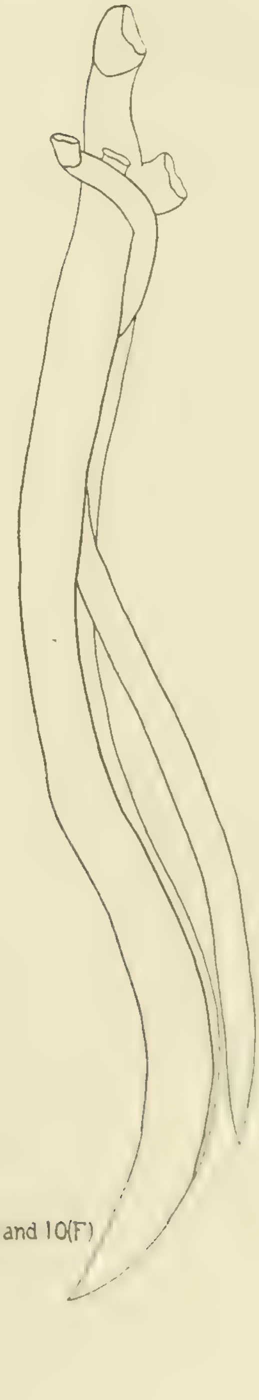
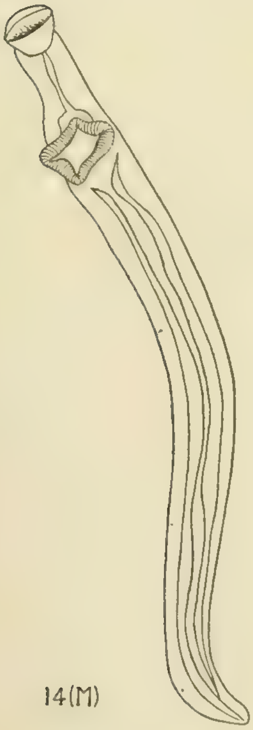
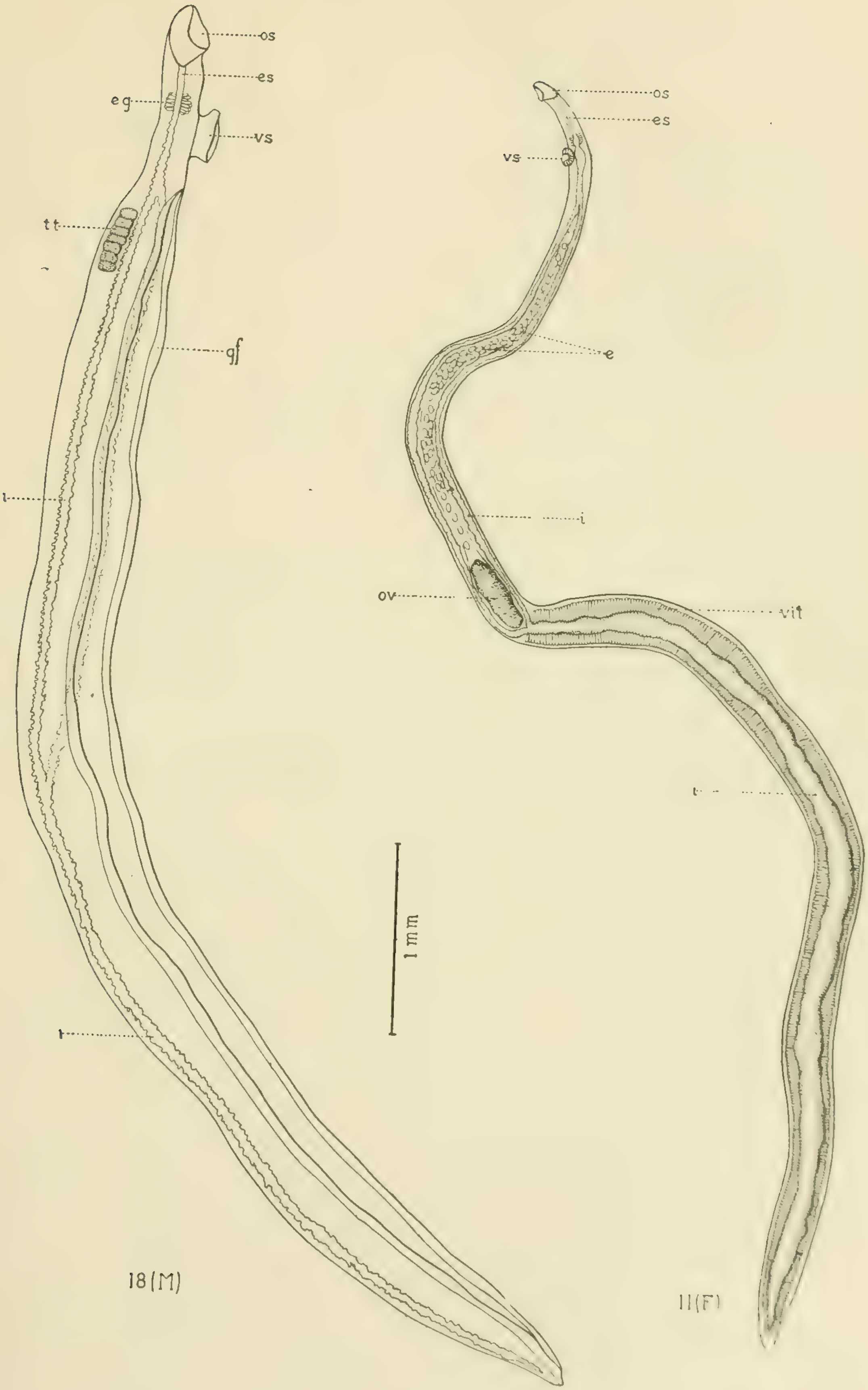


17(M) and 10(F)



14(M)

PLATE III.



18(M)

11(F)

PLATE IV.

Estratto dalle "Ricerche di Biologia didicate al Prof. Alessandro Lustig nel 25° anno del suo insegnanmento universitario," 1914, 13–42, 3 pls.

FUJINAMI, A., AND NAKAMURA, H.

1909. Course of infection of Schistosoma japonicum. Development of the parasite, and animals suffering from the disease. (Japanese.) Mitt. Med. Ges. Kyoto., VI, No. 4.

1910. Pathological changes of Schistosomiasis in Egypt, and a demonstration of S. japonicum in its youngest stage. (Japanese.) Mitt. Med. Ges. Kyoto., VII, No. 3.

1911. Prophylaxis of Katayama disease, and investigations on method of infection. (Japanese.) Chugai Iji shimpo. No. 753.

1911a. A demonstration of specimens showing the development of S. japonicum. (Japanese.) Byo ri Gaku Kaishi, I.

FUJINAMI, A., TSUCHIYA, I., AND MIYAGAWA, Y.

1916. Concerning Japanese Schistosomiasis. (Japanese.) Nisshin Igaku, VI, No. 1.

KATSURADA, F.

1914. Schistosomiasis japonica. Centralbl. f. Bakt. Parasit. u. Infek. 1 Abt. Orig., LXXII, 363-379, 2 pls. and 2 figs. in text.

KATSURADA, F., AND HASEGAWA, T.

1910. Bemerkungen zur Lebensgeschichte des Schistosomum japonicum Katsurada. Centralbl. f. Bakt. Parasit. u. Infek. I Abt. Orig. LIII, 519-522, 1 fig. in text.

LEIPER, R. T.

1915. Report of the results of the Bilharzia Mission in Egypt, 1915, III. Development. Jour. Roy. Army Med. Corps, XXV, 253-267.

LUTZ, A.

1919. Schistosomum Mansoni and Schistosomiasis observed in Brazil. Memorias do Institudo Oswaldo Cruz, XI, No. 1, 109-140, 6 pls.

MATSUURA, U.

1909. On the relationship of an endemic dermatitis to Schistosomiasis, and the entrance of the parasite into the human body. (Japanese.) Mitt. Med. Ges. Kyoto. VI, No. 4.

MIYAGAWA, Y.

1912. Ueber den Wanderungsweg des Schistosomum japonicum von der Haut bis zum Pfortadersystem, und über die Körperkonstitution der jüngsten Würmer zur Zeit der Hautinvasion. Centralbl. f. Bakt. Parasit. u. Infek., 1 Abt. Orig., LXVI, 406-416.

1912a. Experiments in regard to the infection with Schistosoma japonicum through the mouth of the final host. (Japanese.) Tokyoer Med. Wochenschr. No. 1766.

1913. A contribution to the knowledge of the migratory course of S. japonicum in the body of its final host. (Japanese.) Tokyoer Med. Wochenschr. No. 1833.

1913a. On the cercaria of S. japonicum and the young worm at the time of infection. A contribution to the knowledge of prophylaxis of the disease. (Japanese.) Iji shimbun. No. 890-891.

1913b. Ueber den Wanderungsweg des Schistosomum japonicum durch Vermittelung des Lymphgefässsystems des Wirtes. Centralbl. f. Bakt. 1 Abt., Orig., LXVIII, 204–206.

1916. Concerning Japanese Schistosomiasis. II. Aetiology. (Japanese.) Nisshin Igaku, VI, No. 1. (See Fujinami, Tsuchiya, and Miyagawa, 1916.)

NARABAYASHI, H.

1914. On the migratory course of Schistosoma japonicum in the body of the final host. (Japanese.) Kiyoto Igaku Zasshi, XII., No. 1.

1916. A contribution to the knowledge of Schistosomiasis in Japan. (Japanese.) Mitt. Med. Ges. Kyoto, XIII, Nos. 2–3.

SUYEYASU, Y.

1919. On the migratory course of Schistosoma japonicum in the body of the final host. Abstract of a paper given at the 9th meeting of the Japanese Pathological Society. (Japanese.) Igaku Chuo Zasshi, XVII, No. 6.

TANABE, K.

1919. A contribution to the knowledge of the morphology and development of Schistosoma japonicum (Japanese.) An abstract of a paper given before the Japanese Pathological Society. Igaku Chuo Zasshi, XVII, No. 6.

TSUCHIYA, I.

1910. On the experimental therapeutics and the course of infection of Japanese schistosomiasis. Tokyoer Med. Wochenschr. No. 1702.

PRENATAL INFESTATION WITH PARASITIC WORMS *

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In the literature there are few records of prenatal infestation with parasitic worms. I have found indisputable records of such infestation in sheep with the lung worm, *Dictyocaulus filaria,* and in man with the hookworm and the Oriental blood fluke, *Schistosoma japonicum.* Since the larvae of a number of the parasitic worms of man wander widely in the body before finding their final location, and are in many cases carried in the blood stream, prenatal infection might be expected to occur often. It seems probable to me that such infestation is not so rare as the small number of case records would suggest, and that the small number of records is due to a failure to look for infestation in very young infants. It is a fact that where fecal examinations for hookworm disease are carried on, examinations are rarely if ever made of very young babies. Therefore it seems worth while to call the attention of physicians to the possibility of prenatal infestation in man by certain species of parasitic worms, with the hope that some of those who are carrying on work in places where parasitic worms are frequent will look carefully into the matter. Howard,[1] in reporting a case of prenatal hookworm infestation, has already emphasized the importance of further investigations on prenatal infestation in hookworm disease. In the present paper I will outline the cases in which I have found reported prenatal infestation with parasitic worms, and will call attention to those parasitic worms of man in which such infestation would be possible on account of the character of the life cycle.

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[1] Howard, H. H.: Prenatal Hookworm Infection, South. M. J. **10**: 793-795 (Oct.) 1917.

The species in which prenatal infestation has been most carefully worked out is *Schistosoma japonicum*, the Oriental blood fluke. Since I have not found this work taken up outside the Japanese[2] language, I will discuss it here in some detail. Fujinami and Nakamura,[3] in 1911, reported the finding of *Schistosoma japonicum* in the fetus of a dog suffering from schistosomiasis. Narabayashi,[4] in 1914, caused pregnant animals to become infested with the cercariae of *S. japonicum*. In one dog thus infested he found the parasites in five out of seven embryos. In a later series of experiments, Narabayashi[5] was able to find fourteen young specimens of *S. japonicum* in the placentas of two pregnant guinea-pigs that had been experimentally infested. To find these worms it was necessary for him to examine about 20,000 sections of the placenta. He found the young schistosomes most frequently in the blood vessels running from the placenta to the embryo. He concluded, therefore, that the larvae of *S. japonicum* in prenatal infestation are carried to the placenta in the blood stream, migrate through the thin membrane separating the circulation of the mother from that of the fetus, and are carried to the embryo by its own blood vessels. The shortest time after infestation in which the worms were found in the fetus was seventeen hours. This author[5] also examined the feces of twenty-two new-born babies in an endemic area and found the eggs of *S. japonicum* in three. The mothers gave a history of having worked in the rice fields during their pregnancy.

Neveu-Lemaire[6] reported two cases of prenatal infestation in lambs with the common lung worm, *Dictyocaulus filaria*. In the first case the adults of this species were found in the trachea of a lamb that died four days after birth, and in the other they were found in the trachea of a fetus taken from a mother heavily infested with the parasites.

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2. The Japanese investigations on this subject were made available to me through the help of Dr. Sadamu Yokogawa, professor of pathology of the Medical School of Formosa.

3. Fujinami, A., and Nakamura, H.: On the Prophylaxis of Schistosomiasis and Some Investigations on Infection with This Disease (Japanese), Chugi Iji Shimpo, 1911, No. 753.

4. Narabayashi, H.: On Prenatal Infection with Schistosoma Japonicum (Japanese), Verhandl. d. jap. path. Gesellsch. (Japanese) **4,** 1914.

5. Narabayashi, H.: A Contribution to the Knowledge of Schistosomiasis in Japan (Japanese), Kyoto Igaku Zassi **13,** Nos. 2, 3, 1916.

6. Neveu-Lemaire: Strongylose bronchique congénitale du mouton. Compt. rend. Acad. d. Sc. **154:** 1211-1312, 1912.

Howard,[1] in 1917, reported an undoubted case of prenatal infestation with hookworm in a negro child in British Guiana. The hookworm eggs were found in the feces of the child fourteen days after birth, which is entirely too short a time for the development of the hookworm, even if infestation after birth had been possible. The mother had a severe case of hookworm disease and gave a history of ground itch during pregnancy. Howard also notes that in 1916 there was called to his attention a considerable number of cases of hookworm disease in infants aged 1 year or less in Ceylon. He writes in this connection: "In these cases the apparent clinical manifestations were out of proportion to the degree of infection and the length of time these children could have had the disease had they acquired it in the usual way and postnatally."

It would seem possible that prenatal infestation might occur with any parasitic worm in which there is a wandering of the larvae in the body of the final host. The recent discoveries of the wandering of the larvae of *Ascaris lumbricoides* and related forms have suggested that migrations of nematode larvae in their final hosts are more common than has been suspected. These data serve to increase the significance of studies on prenatal infestation. It is worth while in this connection to mention briefly those of the parasitic worms of man in which the life history is such that prenatal infestation is a possibility.

Among the trematodes of man besides those of the schistosome group there is only one, *Paragonimus westermanii,* in which there is wandering in the final host. Since the larvae of *P. westermanii* are much larger than those of the schistosomes, grow rapidly as they wander, and migrate by way of the tissues rather than the blood stream, prenatal infestation with this form, while it might be possible, would seem to be very improbable.

Among the cestodes, the wandering habit is found only in the development of the bladder worm stage. Since the onchospheres are very small and are carried by the blood stream, the infestation of a fetus with the bladder worm stage would not seem to be difficult. In man, the possibility of such infestation would be limited to those species of which the larval stages invade the tissues, as the echinococcus and occasionally the larval stage of *Taenia solium.* I have found no references to prenatal infestation with echinococcus, but there is every reason to believe that such infestation could come about.

It is among the nematodes that prenatal infestation seems to me to offer the most important problem. I believe that with further studies it will be found to be not infrequent with hookworm. Other species in which such infestation might occur on account of what we know of the migrations of the larval stages are *Strongyloides stercoralis, Ascaris lumbricoides* and possibly the various species of the *Filaridae.* It seems possible also that the wandering habit of the larvae is a part of the life cycle of *Trichuris trichiura*[7] and other human nematodes, which would make possible prenatal infestation in these forms also.

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[7] Neshi has recently demonstrated in a paper in Japanese that the larvae of Trichuris depressiscula, the dog whipworm, wander in the final host.

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CHILOMASTIX MESNILI AND A METHOD FOR ITS CULTURE.

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In January, 1920, a chronic case of diarrhea due to a flagellate infection was brought to the notice of the author and upon examination the flagellate was identified as *Chilomastix mesnili*. Attempts to culture this parasitic flagellate proved successful. Observations made from time to time upon the flagellates in both the stools of the patient and the cultures, as well as data derived from a study of permanent preparations, have revealed further information regarding the biology of this organism. It is the purpose of this paper to describe this parasite and its activities and to give a method of culture for the growth of this organism in artificial medium.

Regarding its phylogeny, *Chilomastix mesnili* belongs to the family Tetramitidæ, the order Polymastigina, and the class Mastigophora.

The habitat of the parasite is the small intestine of man. It is often the cause of chronic or intermittent diarrhea. Cases of infection by *Chilomastix* in man have been reported from nearly every locality in the world. Chalmers and Pekkola[1] state that they have always found *Chilomastix* associated with other protozoa and not entirely by itself. But in the case of infection referred to above *Chilomastix* was the only protozoan parasite found. In all probability it is the cause of the patient's diarrhea. No formed stools have been passed since the discovery of the infection 2 years ago which was then thought to be caused by *Trichomonas intestinalis*. In the United States, cases of infection by *Chilomastix mesnili* are by no means rare, for Kofoid, Kornhauser, and Plate[2] found it present in 5.8 per cent of the 1,200 overseas troops which were examined at New York. Of 534 persons examined at Berkeley, California, by Kofoid and Swezy,[3] 28, or 5.3 per cent, were found infected. Many clinicians no doubt have confused *Chilomastix mesnili* with *Trichomonas intestinalis.* This mistake is due to the similarity in movement of the cytostomal flagellum of *Chilomastix mesnili* to the undulating membrane of *Trichomonas intestinalis.*

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[1] Chalmers, A. J., and Pekkola, W., *Chilomastix mesnili* (Wenyon 1910), *Ann. Trop. Med. and Parasit.*, 1918, xi, 213.

Morphology.

The cultivation of *Chilomastix mesnili* in artificial medium has made observations possible from day to day. Under these cultural conditions the flagellates grew and multiplied. Apparently the environment satisfied most of the biological requirements of the parasites. The data given here regarding the morphology and activity of these organisms are based upon direct and continued observations made upon the flagellates under cultural conditions, and also upon a study of permanent preparations made from the cultures and from the feces of the patient harboring the flagellates.

Motile Flagellate.—The location of the various organs in *Chilomastix* will be given as they are seen in a dorsal view, for it is from the dorsal view that convention or custom has always designated the right and left sides of any object.

The best descriptions of the motile or active form of *Chilomastix mesnili* have been supplied by Chalmers and Pekkola[1] and more recently by Kofoid and Swezy.[3] The descriptions given by these two groups of workers differ in some respects; that of Kofoid and Swezy,[3] however, is the more valuable because details of morphology are given which were previously unknown. The description given here corroborates that of Kofoid and Swezy[3] in nearly all details. The differences of interpretation and interpolation of new data regarding the morphology and the activities of the flagellates have come as a result of continued study of the organisms in cultures over a period of 4 months. The cultures were inoculated on January 27, 1920, and were kept alive by subculturing until June 15, 1920, when a mishap to the heating apparatus of the incubator caused it to reach 80°C., which resulted in the death of the organisms.

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[2] Kofoid, C. A., Kornhauser, S. I., and Plate, J. T., Intestinal parasites in overseas and home service troops of the U. S. Army, *J. Am. Med. Assn.*, 1919, lxxii, 1721.

[3] Kofoid, C. A., and Swezy, O., On the morphology and mitosis of *Chilomastix mesnili* (Wenyon), *Univ. California Pub., Zool.*, 1920, xx, 117.

The usual shape of the body of *Chilomastix mesnili* is pyriform with a rounded anterior end and a pointed posterior end terminating in a spine-like process. The latter structure is composed almost entirely of hyaline periplast. The sides begin to taper posteriorly at the middle of the body. This tapering is not uniform for both sides but varied, depending upon the particular state of locomotion the flagellate may be in at any given moment. It is not uncommon to find forms which have the ventrum flattened, and the dorsum arched, so that in profile they resemble a side view of *Giardia intestinalis*. When these particular flattened forms were encountered, the posterior spine appeared as a posterior terminal extension of the ventrum, while in other forms the spine is terminal but more axially situated.

In the stools, the active flagellates have been seen to assume the shape of a sphere, and the spine-like protuberance has been withdrawn by contraction. On two occasions twisted forms have been seen similar to those described by Wenyon and O'Connor,[4] Chalmers and Pekkola,[1] and Kofoid and Swezy.[3] The twisted forms have not been encountered in the cultures. Fortunately, it was possible to observe these atypical flagellates for a considerable length of time and evidence was obtained which may explain their twisted condition.

The twisted state of the flagellate appears to be a temporary condition, at least as far as the flagellates in this particular case are concerned.

Wenyon[5] did not note this twisted condition in his original description of *Chilomastix mesnili* from man, and it was not until later that he and O'Connor[4] described it. Chalmers and Pekkola[1] spoke of this twisted condition as due to "a dorsal twisting of the antero-ventral part, while the posterior part of the body is not affected. This twist naturally gives rise to the appearance of lines and grooves running more or less diagonally backwards across the body." Kofoid and Swezy[3] found that this twisted condition, resulting in a diagonal groove, characterized most of the active forms of *Chilomastix mesnili* and therefore concluded that the groove was a permanent structure. They also believed that in the forms where the groove was not in evidence, it had temporarily disappeared due to the contraction of the body.

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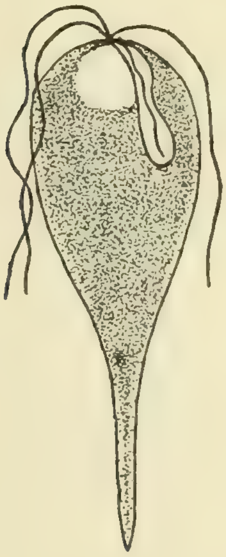
[4] Wenyon, C. M., and O'Connor, F. W., Human intestinal protozoa in the Near East, London, 1917.

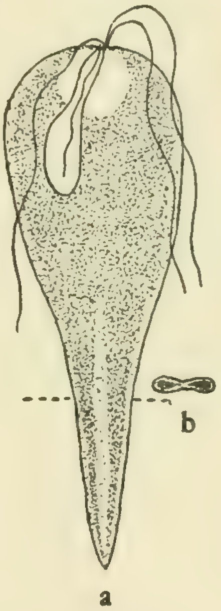
[5] Wenyon, C. M., A new flagellate (*Macrostoma mesnili n. sp.*) from the human intestine with some remarks on the supposed cysts of *Trichomonas*, *Parasit.*, 1910, iii, 210.

Since January, 1920, the author has received fecal specimens once or twice each week from the patient infected with *Chilomastix mesnili*. As has been said before, on only two occasions were the twisted forms encountered. On these occasions it was also found that the flagellates were definitely changed in shape. The anterior end was rounded as usual but the posterior half of the body was broadly flattened from side to side. A dorsal view showed the body almost truly pyriform with uniformly tapering sides (Text-fig. 1). A lateral view resembled the side view of a tadpole, for the posterior end possessed a broad flattened tail instead of a pointed spine (Text-fig. 2, *a*). On closer observation it was seen that the tail contained two rib-like extensions of the granular endoplasm running out into the hyaline periplast of the ectoplasm. These two ribs of endoplasm were united anteriorly where they joined the mass of endoplasm of the anterior portion of the body, but they began to diverge as they proceeded posteriorly and became separated by a thin membrane-like structure made only of the periplast. The area of the tail, constituted only of the membrane of periplast between the two ribs of endoplasm, was thinner than the adjacent areas occupied by the endoplasmic ribs, which were also surrounded by periplast. This condition resulted in the formation of a straight groove on each side of the body between these extensions of the endoplasm (Text-fig. 2, *b*).

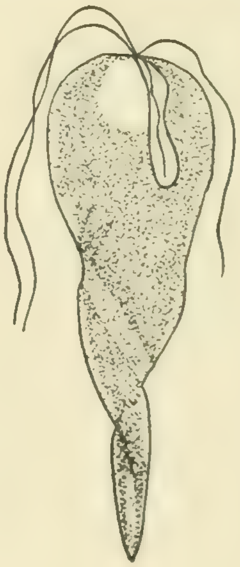
These forms, in addition to being laterally flattened, assumed the twisted condition both when at rest and during locomotion. They possessed definite grooves running diagonally and posteriorly from the left to the right side (Text-fig. 3). To all appearances these flagellates were similar to the twisted forms described by Wenyon and O'Connor,[4] Chalmers and Pekkola,[1] and somewhat like those described by Kofoid and Swezy.[3] In view of the presence of the laterally flattened forms which showed grooves in the posterior region, but were not twisted, it seemed plausible that the grooves of the twisted forms were identical with those of the flagellates which were not twisted, and that this state was a temporary condition brought about by some factor which accounted for the change in form of the flagellates. The flagellates which were not twisted but were flattened may either have recovered from the twisted condition or not as yet become so transformed. The temporary nature of the twisted condition of the flagellates of this particular case of infection seems all the more substantiated since it was never observed in the flagellates which have been growing and reproducing in cultures for over 4 months.



Text-Fig. 1.



Text-Fig. 2.



Text-Fig. 3.

Text-Fig. 1. *Chilomastix mesnili*, dorsal view, unstained. Posterior region compressed. × 3,300.

Text-Fig. 2. *Chilomastix mesnili*, unstained. (*a*) Lateral view showing compressed tail; (*b*) cross-section of tail to show grooves. × 3,300.

Text-Fig. 3. *Chilomastix mesnili*, dorsal view, unstained. Twisted form showing diagonal groove. × 3,300.

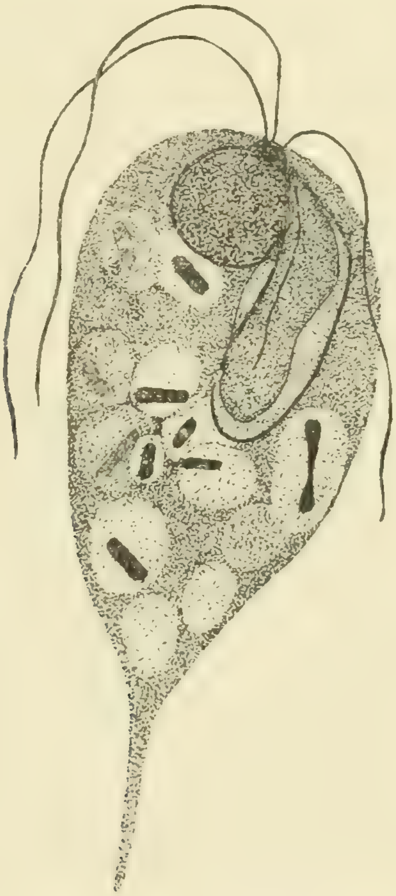
The motile organisms observed in this case measured 3 to 19 microns in length and 2 to 9 microns in width. Most forms in the stool were 8 to 14 microns in length. The flagellates possessed three anterior flagella and a cytostomal flagellum situated in the oral cavity, or cytostome. In a dorsal view (Text-fig. 4) the cytostome is located on the ventrum and the right side, extending backward a distance of one-third to one-half the length of the body. The nucleus is round, and located near the anterior border of the body, to the left of the cytostome and dorsal to it. In unstained specimens it appears somewhat refractile. The cytoplasm is granular and generally contains large vacuoles, in many of which was a bacterial cell which had been previously ingested as food. At the times when binary fission is in process a tendency probably exists on the part of the flagellate to desist from eating, for in many of the forms undergoing division no vacuoles containing bacteria were seen.

In locomotion, *Chilomastix mesnili* is propelled forward and spirally in a jerky manner by the action of its flagella. The two left anterior flagella, as Kofoid and Swezy[3] have pointed out, beat backward and against the left side of the body, and the other anterior flagellum beats backward against the right side of the body. The motion of these flagella is undulatory, the ripples starting at the point of origin of the anterior flagella and proceeding posteriorly. These waves of contraction diminish as they progress distally along each flagellum.

The cytostomal flagellum lies within the cytostome and forms an undulating membrane by adhering to the inner portion of the cytoplasmic lip or membrane which is also within the cytostome (Text-fig. 4). This flagellum is probably not involved in any purpose of locomotion but functions chiefly as a means of obtaining food. Gäbel[6] and Chalmers and Pekkola[1] noted that the flagellum may not always form an undulating membrane but may act as a free flagellum. In the writer's observations upon living forms thus far the flagellum has not been seen as a free whip but always combined with the thin cytoplasmic lip to form an undulating membrane. In the flagellates more or less quiescent, the action of this cytostomal undulating membrane was easily observed. The undulating waves of contraction began at the anterior end of the cytostome at the place of origin of the cytostomal flagellum and proceeded posteriorly to the end of the cytostome. The initial wave of contraction of the undulating membrane was very large, for at times the membrane was pushed outward from the cytostome 3 to 4 microns. The amplitude of this first wave of contraction diminished as the wave progressed posteriorly. The action of the undulating membrane had the effect of

[6] Gäbel, M., Zur Pathogenität der Flagellaten, *Arch. Protistenk.*, 1914, xxxiv, 1.

reaching out into the surrounding medium and literally scooping the bacteria toward the cytostome. In this way food was brought in reach of the cytostome. The anterior flagellum which beat backward against the right side was very close to the cytostome and aided materially in setting up a current in the medium which helped to bring the food in proximity to the cytostome



Text-Fig. 4. *Chilomastix mesnili*, dorsal view, stained. Taken from culture. × 4,400.

The cytostome has had holdfast properties attributed to it by Kofoid and Swezy.[3] No doubt because of its size and morphology it may serve such a purpose, but in the study of the flagellates in the cultures, as well as in fecal specimens, the posterior spine or protuberance was used for this purpose almost entirely.

When the flagellate was feeding, it inserted this posterior spine into a mass of bacteria, while its body remained free from any encumbrance. In this position the flagellate anchored itself and proceeded to rotate, using the posterior spine as the holdfast organ. On such occasions one could watch the undulating membrane as it wafted bacteria into the cytostome. Not all the bacteria which were taken into the cytostome were ingested, for many of them could be seen escaping in a current in the medium directed away from the cytostome. It is very likely that the flagellate exercised a selective sense in the choice of its food.

The finer details of morphology have been described by Kofoid and Swezy.[3] They have shown *Chilomastix mesnili* to possess an elaborate neuromotor apparatus. The author has been able to verify the finding of the structures described by these workers, but because the finer morphological structures which only become apparent in properly prepared, stained specimens have received a full description by the above investigators, brief reference is made to them in this paper.

The nucleus is round and possesses a distinct membrane (Text-fig. 4). It is located near the anterior border of the body to the left of, and dorsal to the cytostome. The chromatin may be distributed in a variety of ways, but it is often in the form of two terminal masses lodged against the nuclear membrane with a small eccentrically placed karyosome, or broken up into chromidia distributed diffusely throughout the nucleus. The chromatin was also resolved into a definite spireme strand, which in some forms had later segmented to form chromosomes.

A very small centrosome is located at the anterior pole of the nucleus (Text-fig. 4). It is often concealed by a larger chromatin-staining mass, the blepharoplast complex. As Chalmers and Pekkola,[1] and Kofoid and Swezy[3] have shown, there are three blepharoplasts which are only seen as separate granules in well decolorized preparations. The primary blepharoplast lies closest and anterior to the nucleus. It gives origin to the pair of anterior flagella which beat backward against the left side of the body. The secondary blepharoplast lies farther to the right side and gives rise to the right anterior flagellum. Kofoid and Swezy[3] found that this secondary blepharoplast also gives rise to the parastyle, a slender, slightly curved rod, which extends posteriorly in the left wall of the cytostome. The tertiary blepharoplast lies at the anterior end of the cytostome and gives rise to the cytostomal flagellum, the peristomal fiber, and the parabasal. All the blepharoplasts are joined together in series with the centrosome and often with the anterior terminal mass of chromatin of the nucleus by fine rhizoplasts. In many forms the primary and secondary blepharoplasts are fused into a single large granule located against the nuclear membrane at the anterior pole of the nucleus (Text-fig. 4). This double blepharoplast by virtue of its position often obscures the centrosome.

The peristomal fiber borders the inner free edge of the membranous lip of the cytostome. It is a fine fibril originating from the tertiary blepharoplast. The cytostomal flagellum when in action appears to adhere to the inner portion of this loose membranous lip to form an undulating membrane within the cytostome, or oral pouch. That this is the case is evidenced by the location of the cytostomal flagellum lying along the side of, and adhering to the inner portion of this lip in many stained specimens. Furthermore, in moribund flagellates it is the inner portion of the lip which is seen to push out for a great distance to begin a wave of contraction which lessens and dies out as the contraction moves posteriorly. In other cases the cytostomal flagellum may be seen to lie on the floor of the cytostome (Text-fig. 4) or at times just under the inner portion of the peristomal fiber.

The parabasal is a large chromatin-staining rod extending from the tertiary blepharoplast along the right side and around the posterior end of the cytostome. In some forms it appears to surround completely the cytostome. However, Kofoid and Swezy[3] found that in such cases, if the preparations have been well destained, this rod is not continuous but is composed of two elements, the parabasal, extending from the tertiary blepharoplast along the right side and around the posterior end of the cytostome, and another rod, the parastyle, arising from the secondary blepharoplast and extending along the left side of the cytostome to within a very short distance of the posterior end of the parabasal. Chalmers and Pekkola[1] believed the parabasal was a continuous rod arising from the secondary blepharoplast and returning to it after it had run a circuitous course around the cytostome.

Kofoid and Swezy[3] have shown the homology which exists between the peristomal fiber and parastyle of *Chilomastix mesnili* with the peristomal fiber and the right axostyle of the right half of *Giardia*. There is good morphological evidence to draw a homology between the peristomal fibers of *Chilomastix* and *Giardia*, and likewise between the parastyle of the former with the right axostyle of the latter organism, but there remains the problem of finding a homologue in *Giardia* for the parabasal of *Chilomastix*. It is more than likely that the parabasals of *Giardia* differ so much in structure and chemical constitution that they cannot be considered homologues of the parabasal of *Chilomastix*. On the other hand, Chalmers and Pekkola[1] showed the parabasal of *Chilomastix* to be homologous to the parabasal of *Trichomonas*, since in both these organisms this rod has the same morphological relation to the undulating membrane. In view of these relations existing between the structures of *Chilomastix*, *Trichomonas*, and *Giardia*, *Chilomastix* may be said to occupy a position midway between the trichomonad flagellates and members of the genus *Giardia*. *Chilomastix* may have resulted as an offshoot from the trichomonad flagellates, and, as Kofoid and Swezy[3] have pointed out, it may be the form from which *Giardia* and other Hexamitidæ have originated.

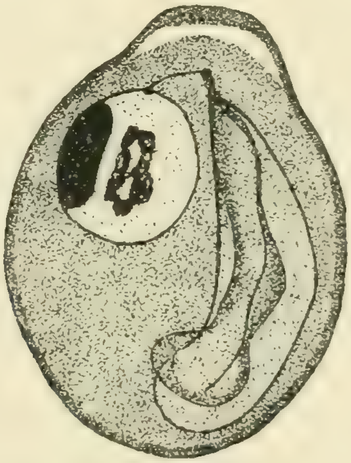
Cysts.—The cysts of *Chilomastix mesnili* are usually found in varying numbers along with the active flagellates. However, they may be entirely absent from a fecal specimen containing an abundance of motile organisms. This fact is evidence of encystment being an irregular if not a cyclic process dependent perhaps on many causes. Wenyon and O'Connor,[4] in the case of a patient undergoing treatment for *Entamœba histolytica*, record the absence of the cysts of *Chilomastix* for 20 days; the flagellates, however, were present on 13 days of this period. In the fecal samples received for examination once or twice each week since January, 1920, the cysts have been absent on only a few occasions.

The cysts are usually somewhat lemon-shaped, with one end rounded and the other end terminating in a slight protuberance (Text-fig. 5). Occasionally they are ovoid and lack the terminal process. In size, the cysts measure 4.5 to 6 microns in width and 7.6 to 9 microns in length. Wenyon and O'Connor[4] record cysts with a length of 10 microns.

The wall of the cyst is transparent and of uniform thickness except in the vicinity of the terminal elevation where it is thicker than at other places.

The cytoplasm within the wall of unstained cysts is refractile and structureless except for the presence of a few bright spots. With favorable lighting facilities for the microscope, a dull brownish area can be seen which is the nucleus. There is a space between the cytoplasm and the cyst wall immediately back of the terminal protuberance.

In stained specimens, the cytoplasm appears to be very finely granulated. At one side toward the anterior end is a large round or ovoid nucleus. The chromatin is often distributed in the form of one large karyosome with accessory chromatin matter, or as a central karyosome with one or two terminal masses, or broken up into chromosomes. Kofoid and Swezy[3] have described in detail the mitosis which takes place within the cyst. These investigators were the first to find cysts containing two nuclei and two sets of neuromotor apparatus. The karyosome of the nucleus is often connected by a rhizoplast with the centrosome on the nuclear membrane at the anterior pole of the nucleus. The centrosome is in turn connected with the blepharoplasts by fine rhizoplasts (Text-fig. 5). In many cysts, as in the case of the motile flagellates, the distinct entity of all three blepharoplasts cannot be made out, only two masses being apparent. The primary and secondary blepharoplasts have become united into a single large granule. The tertiary blepharoplast remains separate at the anterior end of the cytostome.



TEXT-FIG. 5. *Chilomastix mesnili* cyst, stained. × 4,400.

The entire structure of the neuromotor apparatus of the motile organism is preserved and carried over into the cyst. It is possible in properly stained specimens to locate the peristomal fiber, the parabasal, the parastyle, and the cytostomal flagellum. In many cysts, however, some members of this fibrillar apparatus are not discernible. The failure to see these structures may be due to imperfect fixation and decolorization after staining or the structures may have actually disintegrated.

Method of Culture.

Medium.—Less difficulty was experienced in culturing *Chilomastix mesnili* in artificial medium than was anticipated. There were only two fundamental substances used in the preparation of the culture medium; namely, blood serum and a physiological solution. Three kinds of blood serum, horse, sheep, and human, were combined with varying proportions of either Ringer's or Locke's solution. In the attempt to find a suitable medium, the proportions of blood serum and the physiological solutions were varied from equal parts of both to one part of blood serum and two to seven parts of the Ringer's or Locke's solution. But after trying out these various combinations, it was found that the flagellates grew best and multiplied in number in a medium containing the proportion of one part of human blood serum to four parts of Locke's solution. There were limited growth and reproduction in a medium made from one part of either horse or sheep serum to four parts of Locke's or Ringer's solution. But a culture medium containing Ringer's solution did not give as satisfactory results as one containing Locke's solution, and human serum promoted better growth than either the sheep or horse serum.

The human serum[7] comprised the unused portions of the samples sent in to the laboratory for the Wassermann reaction. Both negative and positive sera were used, and they were equally satisfactory.

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[7] This serum was received in a sterile condition from the Department of Bacteriology of the Baltimore City Board of Health through the kindness of Dr. William R. Stokes.

The Locke's solution was made up as follows:

Sodium chloride	0.9	gm.
Calcium chloride	0.024	"
Potassium chloride	0.042	"
Sodium bicarbonate	0.02	"
Distilled H_2O	100.00	cc.

To this solution 0.25 gm. of dextrose was added, and it was then sterilized. After the sterilization of the Locke's solution the human serum was mixed with it in the proportion given above, care being taken to prevent any contamination of the resulting medium. The culture medium was distributed in 5 cc. amounts in small test-tubes and these were incubated at 37°C. over night and examined the next morning. The tubes which showed any evidence of bacterial growth were discarded. The medium generally had a clear cream-colored appearance unless blood cells were present in the blood serum, in which case the medium was reddish in color until the blood cells sank to the bottom of the tube. If in the samples of blood a slight amount of hemolysis had taken place, the serum was tinted red by the hemoglobin. Culture medium containing either red blood cells or hemoglobin as the result of the hemolysis of the cells did not prevent the growth and propagation of the flagellates but at times it appeared more favorable for their growth than culture medium devoid of these substances.

The reaction of several batches of the culture medium varied from neutral to 0.4 per cent alkaline to phenolphthalein. A reaction of 0.2 per cent alkaline usually resulted from the mixture of the human blood serum and Locke's solution.

Technique.—It was found advisable to wash a small portion of the stool containing the flagellates before inoculating the culture medium, in order to get rid of a superabundance of bacteria, fecal debris, and products of chemical decomposition which in the beginning might prove inhibitory to the growth of the organisms in artificial medium. Accordingly a loop of fecal material containing numerous flagellates was washed by stirring it in with about 2 cc. of warm sterile normal saline solution and then taking a couple of drops of this suspension containing the flagellates and mixing it with another small amount of normal saline solution. This procedure was followed a third time if it was found that the second resulting suspension was very turbid from the presence of much fecal detritus and numerous bacteria. A couple of drops from each of the above suspensions were inoculated into each of several tubes of culture medium and incubated at 37°C.

On the following day the tubes were examined. Some showed an absence of flagellates; others showed the presence of *Chilomastix* but in small numbers. Transplants were made from the positive cultures by transposing several loops of medium containing the flagellates. On the 2nd day after the original inoculation the positive tubes showed an increase in the number of organisms. Their number was increased even more the following day, at which time more transplants were made. The first inoculation was begun on January 27, 1920, and transplants were made at various intervals. The flagellates were kept in this artificial medium from the above date to June 15, 1920, when an accident to the heating device of the incubator caused the temperature to rise to 80°C., which killed the flagellates.

Other attempts to grow *Chilomastix* taken from the feces in the Locke-human serum medium have proved successful and have demonstrated the favorableness of this artificial medium as a habitat for these parasitic flagellates of man.

Growth in Cultures.

Period of Lag.—The life of a culture, as denoted by the presence of living flagellates, varied in length from 2 to 10 days. The average length of life for each culture was about 6 to 8 days.

The transplants from old to new cultures were usually made in the morning. Such inoculations into new cultures always carried over numerous flagellates and bacteria from the old culture. Little or no growth was noticeable during the early hours of incubation of the new cultures. A period of lag, similar, perhaps, except in length, to the lag phase in fresh cultures of bacteria, apparently characterized the growth of the flagellates during the early hours of the life of a culture. The evidence for a lag phase in the growth of these protozoa in cultures was obtained from the observation of cultures at frequent intervals during the first 22 hours of incubation.

A culture of *Chilomastix* which was transplanted at 9 a.m. and kept in the incubator at 37°C. was observed hourly throughout the same day and night up to 7 a.m. of the following day. Likewise vaseline preparations on depression glass slides were inoculated at the same time as the above culture and observed at the same intervals. Several loops of the culture were withdrawn for observation at each interval of an hour. The vaseline preparations permitted observation of their entire contents, since these preparations had been made upon glass slides which were easily manipulated upon the stage of the microscope.

The first signs of dividing organisms were noticed at 10 p.m. and the flagellates were scarce in number. At midnight, 15 hours after inoculation, the dividing forms were encountered more frequently, and dividing flagellates were in evidence at all subsequent examinations. The number of dividing forms seen during any one observation never exceeded six, but in view of the small volume of several loops of medium taken for each observation, the rate of reproduction was high. The period of lag, in the case of this particular culture, was from 9 a.m. until 10 p.m., or 13 hours in length. During this period of lag it is more than probable that multiplication of the organisms was going on, but the organisms were so few in number that they were missed in the small sample of medium taken for observation. In view of this circumstance it cannot be definitely concluded that the period of lag is 13 hours in length, for with more experiments it may be found to vary considerably.

Presence of Bacteria.—During the 1st day in the life of a culture, much gas was produced by the bacteria. A slight scum appeared on the surface of the medium and small bacterial clumps were present at the bottom of the culture. The scum became thicker and the precipitation of bacteria forming clumps became greater during the subsequent days of growth in the cultures.

Most of the bacteria present in the cultures were identified and were chiefly members of the colon-typhoid group. The bacteria identified were *Bacillus coli communis*, *Bacillus coli communior*, *Bacterium aerogenes*, *Bacillus alkaligenes*, and *Bacillus proteus*. *Bacillus subtilis* was found in a few cultures; whether or not it promoted or inhibited the growth of the protozoa was not determined. It was not present in early cultures and no transplants were made from cultures containing this bacillus.

At the death of a culture, when all the flagellates had disappeared, the reaction of the medium had been changed by the activities of the bacteria. The reaction at the end was always more strongly alkaline. One would expect that the presence of *Bacillus coli*, *Bacterium aerogenes*, and *Bacillus proteus* would produce a sufficient quantity of acid by the fermentation of the dextrose in the medium to turn the alkaline reaction of the medium in the beginning, to acid, 8 days later. The gas present in the cultures during the first few days after inoculation was the result of the fermentative action of *Bacillus coli*, *Bacillus proteus*, and *Bacterium aerogenes*. During this time it is probable, for it was not determined, that the reaction might have been slightly acid, but the effect in such a case was only temporary as the reaction had become alkaline in the end. The increase in alkalinity might have been due to the presence of *Bacillus alkaligenes*, for it was found that this organism was the most predominant of all the bacteria, as evidenced from the number of colonies on an agar plate planted from a culture in which all the flagellates had disappeared.

Most of the flagellates were found feeding upon the bacterial clumps at the bottom of the culture. They were so numerous on the 3rd or 4th day after inoculation of the cultures that they often appeared as a compact mass, with their bodies touching each other and showing little or no progressive locomotion. The presence of most of the flagellates at the bottom of the culture would indicate their preference for a medium in which the oxygen content was somewhat reduced.

Size of Flagellates.—The size of the flagellates in the cultures ranged from 3 to 18 microns in length and from 2 to 9 microns in width. During the first 3 or 4 days when the flagellates were numerous, there was a great number of small forms measuring from 3 to 8 microns in length. The small size of these forms was undoubtedly due to the rapid rate of reproduction which took place during the 1st day especially, and later, during the 2nd, 3rd, and 4th days. This rapid proliferation on the part of the flagellates did not permit of the organisms reaching their full growth previous to another division. Many of the small forms were probably the products of multiple fission. Near the end of the life of the culture when the number of flagellates was small, the organisms were apparently normal in size; *i.e.*, about 8 to 14 microns in length.

The difference in the average size of the flagellates in the cultures and those obtained directly from the feces was due apparently to a more rapid rate of multiplication in the culture than in the intestine of man. The factors which might have caused this great rate of reproduction in the cultures had perhaps a better opportunity of influencing the growth of the protozoa in the test-tube than in the intestine of man, since they were concentrated into a smaller area. And furthermore, they accumulated more rapidly in the cultures than in the human intestine where digestion was accomplishing changes in the intestinal medium of the organisms.

Cysts.—Thus far, no cysts have been found in any of the cultures. Whether encystment was inhibited by the lack or presence of certain substances in the medium was not ascertained. Skatole has been known to cause encystment of amebæ, but as yet no experiments have been made with the use of this product of protein decomposition to induce the encystment of *Chilomastix*.

Rapidity of Growth.—A detailed study made on five different cultures of *Chilomastix* furnished data as regards the growth and multiplication of the flagellates. The activities of the flagellates in these cultures were not unlike those in all the cultures made of *Chilomastix*.

The number of flagellates per cubic centimeter of culture medium was computed for each day during the lifetime of the five cultures. The method of estimating the number of flagellates per cubic centimeter was as follows: Each culture was shaken vigorously for 1 minute previous to examination in order to distribute the organisms uniformly throughout the medium. Only a sufficient amount of the medium containing the flagellates was placed in the counting chamber of a hemacytometer to prevent an overflow into the moats of the chamber. Immediately before placing the flagellates in the counting chamber, a minute drop of saturated corrosive sublimate solution was placed on the floor of the counting chamber and mixed with the culture medium as both were drawn into the chamber by capillary attraction. The drop of saturated corrosive sublimate solution was used to kill the flagellates, thus preventing their moving about over the ruled area and avoiding more than one count. This minute drop of corrosive sublimate solution increased the percentage of error to some extent, yet the total count of organisms per cubic centimeter as determined by this method can be said to be fairly representative of the actual number. All the flagellates were counted which were found lying within the confines of the total ruled area of the counting chamber and by simple calculation the number of flagellates in a cubic centimeter was computed.

Each of the five cultures containing 5 cc. of medium with a reaction of 0.1 per cent alkaline to phenolphthalein was inoculated on May 19, 1920. Each culture was inoculated with three loops of medium from another culture having about 200,000 organisms per cubic centimeter. In a representative loopful, about 250 flagellates were counted, hence it follows that each culture received about 750 organisms, or about 150 flagellates for each cubic centimeter of the new culture.

The number of organisms per cubic centimeter from day to day for all five cultures is given in Table I. The per cent increase in the number of flagellates is also given for each day.

On the following day, May 20, there was evidence of the great fertility of these flagellates in the great increase in their number. Culture 3, which contained such a scattering of organisms that they were too scarce to be counted with the hemacytometer, was an exception; but it behaved similarly on the following day. This latter culture showed no apparent increase in the number of flagellates and in all probability contained few more than the original number inoculated. Proliferation had been inhibited or delayed for a period of 1 day. The remarkable fecundity of these flagellates is surprising when one notices in Culture 2 an increase in the number of flagellates from 150 per cubic centimeter to 26,666 per cubic centimeter, an actual increase over the number of May 19 of 17,677.3 per cent. Cultures 1, 4, and 5 also showed remarkable increases in the number of flagellates, Culture 4 being the lowest with an increase of 5,405 flagellates, or 3,603.3 per cent, over the number of the day before. As has been said, the greatest multiplication takes place during the 1st day.

On the next day, May 21, the per cent of increase in the number of flagellates had fallen for Cultures 1, 2, 4, and 5, although the per cent increase for Culture 4 was about the same as that of May 20. Culture 3 showed a large increase from about 150 to 11,111 flagellates per cubic centimeter, an increase of 7,307.3 per cent. Culture 5 had the largest number of organisms per cubic centimeter, which was about 277,777. Culture 2 followed closely.

TABLE I.

Date.	Culture No									
	1		2		3		4		5	
	No. of flagellates per cc.	Per cent.	No. of flagellates per cc.	Per cent.	No. of flagellates per cc.	Per cent.	No. of flagellates per cc.	Per cent.	No. of flagellates per cc.	Per cent.
1920										
May 20	15,555	<10,270.0	26,666	<17,677.3	Few.		5,555	<3,603.3	20,000	<13,233.3
" 21	150,000	<864.3	253,000	<848.7	11,111	<7,307.3	204,444	<3,580.3	277,777	<1,288.8
" 22	255,555	<70.3	702,222	<177.5	293,333	<2,540.0	437,777	<114.1	725,555	<161.2
" 23	280,000	<9.5	300,000	>57.2	368,000	<25.4	524,000	<19.7	365,555	>49.6
" 24	232,222	>17.0	208,888	>30.3	357,777	>2.7	472,222	>9.8	401,111	<9.7
" 25	216,666	>6.6	153,333	>26.5	255,555	>28.5	327,777	>30.5	Dead.	
" 26	24,444	>88.7	Dead.		23,333	>90.8	Dead.			
" 27	Dead.				Dead.					

Cultures inoculated May 19, 1920. Number of flagellates per cubic centimeter and per cent increase recorded for each day. < indicates increase; >, decrease.

On May 22, the per cent increase in the number of organisms dropped again for all the cultures including Culture 3. 3 days after inoculation the maximum number of flagellates per cubic centimeter was reached in Cultures 2 and 5 which was 702,222 and 725,555 respectively. Culture 3 showed a much larger number than the day before, increasing from 11,111 to 293,333 flagellates per cubic centimeter, an increase of 2,540 per cent.

On May 23, Cultures 2 and 5, having reached the maximum number of flagellates per cubic centimeter on the previous day, now showed a marked decrease per cubic centimeter of 402,222 flagellates (57.2 per cent) for Culture 2, and 360,000 flagellates (49.6 per cent) for Culture 5. 4 days after inoculation, Cultures 1, 3, and 4 contained the largest number of flagellates per cubic centimeter, although the number of flagellates for each culture was considerably less than the number attained in Cultures 2 and 5 on the day before. Also the per cent increase in the number of organisms for Cultures 1, 3, and 4 was comparatively low.

On the next day, May 24, Cultures 1, 3, and 4 first began to show a decrease in the number of flagellates, but the decrease, ranging from 2.7 to 17 per cent was much smaller than the initial per cent decrease of Cultures 2 and 5 on the previous day. And on this day, Culture 2 continued to decline in its number of flagellates but Culture 5 showed evidence of some increase, for it gained from 365,555 to 401,111 flagellates per cubic centimeter. Nevertheless, it still showed a 44.7 per cent reduction from the maximum number of organisms of May 22.

On May 25, all the cultures showed a greater falling off in the number of flagellates from the day before, and Culture 5 no longer contained flagellates. A drop of 401,111 flagellates per cubic centimeter to none at all had occurred in the 24 hour interval between examinations.

On May 26, Cultures 2 and 4 were also found to contain no flagellates, while Cultures 1 and 3 had suffered a tremendous loss. The decrease was 88.7 and 90.8 per cent, respectively. These large decreases in the number of flagellates and the per cent were forewarning that the organisms would not be found alive much longer, and on the next day, May 27, all had disappeared.

From the study of these five cultures, it was seen, as it had been noticed in all previous cultures, that the period of the greatest rate of proliferation took place during the 1st day and that the greatest number of flagellates was to be found in the cultures on the 3rd or 4th day. Rarely a second phase of multiplication occurred, and when present it usually came on the 5th day when a marked decrease in the number of organisms had already taken place on the day before. Culture 5 showed evidence of this behavior. The rate of increase in the number of flagellates decreased after the 1st day, becoming less and less up to the 3rd or 4th day. Then a decrease in the number of flagellates occurred, which ended in their disappearance on the 6th, 7th, or 8th day. The rate of increase and decrease in the number of organisms varied for each culture, although they were somewhat similar, in that one could expect an increase during the early days in the life of a culture and a decrease after the 3rd or 4th day. In some instances the rates in per cent of increase and decrease were similar for different cultures. The death and disappearance of all the organisms on the last day were sudden, thousands of flagellates per cubic centimeter being destroyed within a single day.

Food.—The food of *Chilomastix* was comprised chiefly of bacteria although the mineral salts of the Locke's solution, the proteins of the blood serum, and the sugar may also have served to some extent as food and a source of energy. But one cannot observe the flagellates using the latter substances as food as they do the bacteria in the medium. When the bacteria are ingested they may be seen within the food vacuoles of the cytoplasm. The process of ingestion has been described above.

It is probable that the presence of the bacteria whether they were for the purpose of food or not may explain in part some of the more important questions concerned with the life of the flagellates in the cultures.

Fertility and Death of the Flagellates.—No definite evidence has been uncovered as yet to explain the great fertility of the flagellates during the 1st day in a new culture but certain theoretical conclusions warrant consideration. It has already been said that a period of lag preceded the period of excessive and rapid multiplication. This lag period, as yet, has not had its length of time determined, although the first dividing flagellates were found 13 hours after the inoculation of the cultures.

In the growth of bacteria a period of lag has been known for some time past. Lately, Salter[8] working with *Bacillus coli communis* showed that the length of this period was from 3 to 8 hours, depending upon the medium used and the age of the original culture from which subcultures were made. It is probable that the period of lag of

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[8] Salter, R. C., Observations on the rate of growth of *B. coli*, *J. Infect. Dis.*, 1919, xxiv, 260.

Bacillus coli communis is similar in length to the period of lag for other members of the colon-typhoid group in favorable media.

The bacteria present in the cultures of *Chilomastix* belong chiefly to the colon-typhoid group. Since some of these bacteria were used as food, the food supply of the flagellates was scarce during the lag phase of the growth of the bacteria. This scarcity of food supply might have been in part responsible for the period of lag in the growth of the flagellates when little or no multiplication occurred. The bacteria passing through the lag phase then entered upon a period of great multiplication. Within several hours the bacteria increased in number from a few hundred to many million. This great number of bacteria in the cultures furnished adequate food for the flagellates, and it seems only natural that the period of great fertility of the flagellates should now follow in the presence of this food supply.

Chemical decomposition products of bacterial metabolism have been forming in the cultures which have probably altered the culture medium in such a way as to make it most favorable for the growth of the protozoa. Because of these circumstances, it is very likely that the period of great fecundity of the flagellates was dependent to some extent upon the previous period of rapid multiplication of the bacteria, by virtue of which the culture medium was rendered favorable for the growth of the protozoa and an abundant food supply was obtained.

Table I shows that the rate of increase of the flagellates in the cultures began to decline after the 1st day. From the 3rd or 4th day on, the flagellates decreased in number and in most cases all had disappeared from the cultures on the 6th, 7th, or 8th day. This characteristic increase and decrease in the number of flagellates after the 1st day might also have been due to the metabolic relations existing between the protozoa and the bacteria, as appears to have been the case in the explanation of the great fecundity of the flagellates on the 1st day.

The chemical products of decomposition brought about by the metabolic activities of the bacteria accumulated rapidly during the first 24 hours, during which time the rate of multiplication of the bacteria was at its height. The amount of the chemical substances produced within this time was just sufficient to alter the medium to the point where it was most favorable to the proliferation of the protozoa. Following the period of rapid reproduction of the bacteria, their rate of multiplication was slower and more or less constant; nevertheless, the chemical products were being formed and accumulated, but at a slower rate. Furthermore, mortality among the bacteria occurs at a slow rate in the early life of a culture but increases with the age of the culture. So in the culture of *Chilomastix*, as the death rate of the bacteria increased, the decomposition of their bodies produced other substances in the medium which might have been detrimental to the life of the flagellates.

Just as a minimum amount of these products of decomposition might have served to make the culture medium favorable for the rapid multiplication of the flagellates during the 1st day, so, through their accumulation from day to day, an excess of these substances may have become poisonous and thus inhibited the proliferation of the flagellates at a rapid rate after the 1st day. If this were the case, the continued accumulation of these now poisonous products caused a decrease in the number of the protozoa after the 3rd day and ultimately brought about the death and complete disappearance of the flagellates from the culture.

Some experiments have been performed upon amebæ, which, with the use of certain products of protein decomposition, resulted in an increased rate of cell division. The results of these experiments appear to lend evidence in support of the above theory that the chemical products of protein decomposition promoted the proliferation of the flagellates when present in small amounts, and that they became poisonous when present in larger amounts. The action of these substances as found in the experiments upon the ameba has well defined limits within which cell division is induced at a rapid rate, and beyond which death occurs.

The researches of Fantham[9] on *Entamœba coli*[10] showed that tyrosine, an aromatic compound and a product of protein putrefaction, when present to the extent of 0.2 per cent in the culture medium, had the effect of shortening the life cycle, increasing the number of generations in any one culture, and increasing the frequency of binary fission.

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[9] Fantham, H. B., On the amœbæ parasitic in the human intestine, with remarks on the life-cycle of *Entamœba coli* in cultures, *Ann. Trop. Med. and Parasit.*, 1919, v, 111.

[10] Dobell (Dobell, C., The amœbæ living in man, London and New York, 1919) believed that the organism which Fantham cultured was not the parasitic ameba, *Entamœba coli*, but a free living species.

Similarly the work of Cropper and Drew[11] on the cysts of the free living ameba, *Amœba ostrea*, showed that tyrosine (3 per cent) caused excystation in 24 hours; the free forms became larger, and among them were many multinucleate types. Choline, a member of the amine group and a product of the decomposition of amino-acids, showed even stronger powers in inducing cell division than the tyrosine. In one experiment two culture slides, A and B, contained equal volumes of 0.2 per cent choline in distilled water and distilled water, respectively. Upon A were 34 amebæ and on B, 26 amebæ. After 3 days the number of amebæ on A had increased from 34 to 207 and on B from 26 to 88. These investigators concluded that the substances "undoubtedly in certain strengths cause great proliferation of amœbæ," but also that when a certain strength of these substances is exceeded "the amœbæ are poisoned and multiplication is retarded."

In the study of the five cultures given in Table I, the reaction of the medium was changed from 0.1 per cent alkaline to phenolphthalein in the beginning, to 0.3 per cent alkaline at the end when all the flagellates had disappeared. The question arises as to whether or not the change in the reaction of the medium was responsible for the death of the flagellates. The change in the reaction may have been a contributing factor in the death of the flagellates, but at this time it does not seem fair to attach too much importance to this factor, because the flagellates thrived very well in some batches of medium with an initial reaction of 0.3 per cent alkaline to phenolphthalein which became more alkaline at the death of the culture. In view of this evidence it seems more probable that the death of the flagellates was brought about through the poisonous action of the chemical decomposition products of bacterial metabolism and not as a result of a change of the reaction of the culture medium.

In view of this theory, the persistent character of infections of man by *Chilomastix* may be explained in part. The accumulation of the products of protein decomposition does not normally occur in the intestine of man since the act of defecation carries them to the outside. The disappearance of the flagellates from the stools for short or long intervals might be explained by the death of numerous flagellates through the toxicity of the substances of protein decomposition, temporarily accumulated, which in turn have altered the digestive tract to such an extent as to make it an unfavorable habitat. The removal of the substances would bring about a period of great proliferation which would be indicated by the presence of the flagellates in the stools again.

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[11] Cropper, J. W., and Drew, A. H., Researches into induced cell-reproduction in amœbæ, London, 1914.

Reproduction in Cultures.

No detailed account of the methods of reproduction of *Chilomastix mesnili* will be given in this paper because of the inability thus far to study a sufficient number of stained preparations. It is hoped that at a later date this may prove possible. The observations to be recorded at this time were made chiefly upon living organisms, as the dividing forms were encountered during the examination of the cultures.

Binary Fission.—Binary fission was the type of cell division most often encountered, and in all probability it was the chief method of multiplication in the cultures after the 1st day. In the early stages of binary fission, the dividing flagellate was somewhat ovoid in shape, devoid of bacteria in most cases, and marked by the absence of a posterior protuberance. The first stage observed indicated an advanced period of division, for two nuclei were seen as bright refractile areas and a cytostome was present on either side. It could not be definitely determined whether a double set of anterior flagella was present or not, but there appeared to be a cytostomal flagellum for each oral pouch.

As the plasmotomy of the cytoplasm progressed, it was noticed that the plane of division was median and longitudinal. The separation of the two daughter flagellates began by a cleavage of the cytoplasm which proceeded anteroposteriorly. The division was apparently equal for both resulting organisms. The further stages in plasmotomy were aided considerably by the flagellar action of the organisms. The resulting daughter organisms tugged in opposite directions and the terrific lashing activity of the flagella no doubt served to separate the individuals. Just previous to their final separation, the two daughter flagellates were bound together by a fine, terminal strand of cytoplasm composed apparently of only the hyaline periplast. The breaking of this strand set the organisms at liberty, while the resulting portions of the strand became the posterior spine for each flagellate. In one instance, approximately 35 minutes elapsed between the stage wherein plasmotomy had just begun to the final separation of the daughter flagellates.

From some stained preparations, there was noticed the formation of chromosomes, whose number appeared to be four, also the division of the centrosomes with the formation of a paradesmose. The paradesmose increased in length during the separation of the daughter nuclei. No study has been made of the different phases of mitosis, but the observations which have been made thus far have been in part recorded by Chalmers and Pekkola.[1]

Multiple Fission.—Evidence of multiple fission was also noticed in the cultures of *Chilomastix*. It is probable that this method of reproduction was the one most often resorted to during the 1st day, when the rate of multiplication was at its highest. The only flagellates apparently undergoing multiple fission were found during the first 22 hours in a culture which had been observed at intervals of an hour throughout 1 day and night until the following morning. Stained preparations were made, which revealed a few forms possessing four incomplete sets of organella.

In the living forms undergoing multiple fission, as in the case of binary fission, no posterior protuberance was visible. The body of the flagellate was large and spherical, in one instance measuring 19 microns in diameter. These forms did not appear to contain any bacteria, but the cytoplasm was more or less uniform in its granular constitution. The organism exhibited distinct ameboid movements by the bulging out and subsequent withdrawal of considerable areas of its body. The parts of the body which were so pushed out were usually equipped with flagella and a cytostome.

The body of the flagellate was a somatella comprising four zooids. The somatella possessed four distinct cytostomes located in different sectors of the body. Each cytostome had a cytostomal flagellum and nearby there appeared to be a single anterior flagellum. The nuclei were not seen in the living forms, but in the stained preparations the forms undergoing multiple fission possessed four nuclei. The nuclei were located in different sectors also, and near the cytostome of the respective sector.

Conjugation.—Whether or not other observations made upon *Chilomastix* in the cultures are to be interpreted as probable signs of conjugation can only be determined by further investigation. It seems advisable to record the observations of this nature made thus far.

It was noticed in the cultures, chiefly in those which were only 1 to 3 days old, that some of the flagellates were joined together in pairs. But while in this position, each flagellate was very actively beating with its flagella, and the manner of their union was such as even to permit the reversal of their respective positions. In the majority of these cases the common position of their bodies was such that their anterior ends were pointed toward the same direction, and when reversed the anterior end of each flagellate would point in the opposite direction. This reversal of direction occurred for only a moment, after which the original position was assumed again. Such forms were observed for a long time, but they were never seen to separate.

At present in the absence of more evidence from stained preparations one cannot attribute any syngamic significance to these double forms, since no actual transfer of nuclear substance was noticed. The two bodies of the flagellates in union did not apparently fuse together, for a line marking the confines of the cytoplasm of the organisms was always visible at the point of junction.

It must be stated, too, that clumping of the flagellates also took place at the edge of the cover-slip preparation, where the oxygen was more abundant. It does not seem, however, that the union of flagellates in pairs as described above was of the same nature as the clumping of the organisms in aggregates of a dozen and more in the area of the greatest abundance of oxygen. The paired forms were often seen after a sample had been taken directly from the cultures, at which time no clumping of the flagellates had occurred at the edge of the cover-slip.

SUMMARY.

1. Cases of flagellate infection of man by *Chilomastix mesnili* have been reported from nearly every locality in the world. They are fairly abundant in the United States and are often confused with cases of infection by *Trichomonas intestinalis*.

2. The shape of *Chilomastix* is pyriform. The body contains the following structures: nucleus, centrosome, primary, secondary, and tertiary blepharoplasts, parabasal, parastyle, peristomal fiber, ventral cytostome, cytostomal flagellum, three anterior flagella, food vacuoles with bacterial inclusions, and a posterior spine-like process, the hold-fast organ.

3. The twisted shape in some flagellates in this case of infection is a temporary condition. It was seen in flagellates taken from the feces of the patient on only two occasions and was never observed among the cultured forms.

4. The cysts of *Chilomastix* are lemon-shaped and occurred irregularly in the stools of the patient. They have not been encountered thus far in the cultures.

5. *Chilomastix mesnili* was cultured continuously from January 27 to June 15, 1920, in an artificial medium composed of one part of human serum and four parts of Locke's solution with the addition of a small amount of dextrose.

6. Cultures sometimes remained viable for a period of 1 to 10 days, but generally for a period of 1 to 8 days.

7. The flagellates in the cultures increased in number during the 1st, 2nd, 3rd, and often the 4th days, after which there was a decrease until all had disappeared.

8. A period of lag, probably 13 hours in length, and followed by a period of great fecundity, characterized the growth of the flagellates during the 1st day in the cultures.

9. The number of flagellates in the cultures varied considerably and often reached 700,000 per cubic centimeter.

10. The bacteria served in part as the food of the flagellates. The bacterial organisms identified in the cultures were *Bacillus coli communis*, *Bacillus coli communior*, *Bacterium aerogenes*, *Bacillus alkaligenes*, and *Bacillus proteus*. The proteins of the blood serum, the mineral salts, and the dextrose in the medium may also have proved a source of food for the protozoa.

11. The products of bacterial metabolism, resulting from the growth of the bacteria, their fermentative activity, and their death may explain the rate of growth, reproduction, and death of the flagellates in the cultures. A certain amount of the products of decomposition

together with the presence of the bacterial food supply may explain the rapid proliferation of the flagellates during the 1st day and the slower rate up to the 3rd or 4th day. An excess of these products may have inhibited the growth and multiplication of the protozoa, especially after the 3rd or 4th day, and ultimately may have brought about their death and disappearance from the cultures.

12. The initial alkalinity of the medium increased at the death of the culture. It is not believed that the change in alkalinity proved lethal to the protozoa.

13. Binary fission was often observed. The plane of division was median and longitudinal. A paradesmose was formed.

14. Multiple fission also occurred. The flagellate body, ameboid in movement, was a somatella comprised of four zooids. It contained four cytostomes, each with a cytostomal flagellum. The number of anterior flagella was not complete for each zooid.

15. Flagellates, joined in pairs, were often seen during the first 3 days in the cultures. They may represent conjugating forms, but as yet no evidence of the exchange of nuclear substance has been seen.

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EXPERIMENTS ON THE CARRIAGE OF INTESTINAL PROTOZOA OF MAN BY FLIES.*

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PLATES VI TO VIII.

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INTRODUCTION.

The danger to the public health arising from the pernicious activities of the common housefly and its relatives is now well known. Numerous experiments, ably summarized by Graham-Smith (1914), have shown conclusively that flies may be important factors in the spread of such diseases as typhoid fever, paratyphoid, cholera and infantile diarrhea, in which the bacteria which cause the disease are found in large numbers in the feces of the person affected. These experiments have also shown, in great detail, how long these bacteria remain infective when carried by flies.

Besides these bacterial parasites of the human intestine, there are other parasites belonging to the group of the Protozoa. The world war gave a great impetus to the study of these comparatively neglected forms, and they are proving to be far more common in temperate climates than was formerly supposed. Amoebic dysentery, produced by *Entamoeba histolytica*, is the only disease which has been definitely proved to be due to an intestinal protozoan parasite, but it is highly probable that *Giardia* (*Lamblia*) *intestinalis* and perhaps other flagellates will be found capable of producing diarrheal symptoms under certain conditions.

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In the case of the intestinal bacteria carried by flies, it is known that the bacteria are unable to persist indefinitely within the intestine of the fly. Sooner or later they are killed, either by the action of the digestive secretions or by crowding due to the multiplication of the harmless bacteria which normally inhabit the fly's intestine. Although, as will be seen in the next section, considerable work has already been done on the carriage of the cysts of intestinal protozoa by flies, it has not yet been clearly shown whether the cysts pass through the fly and are deposited in its feces entirely unaltered, or whether they, like the bacteria, are ultimately killed by too long a sojourn within the alimentary tract of the fly. The solution of this problem is the main purpose of the present paper.

HISTORICAL.

The first attempts to test by experiment the possibility of the distribution of cysts of intestinal protozoa by flies occurred in 1913. Stiles and Keister (1913) proved that houseflies could carry "Lamblia spores" (the cysts of *Giardia intestinalis*) on their outer surface, and pointed out that, from epidemiological considerations, the carriage of cysts by flies from open privies to kitchens was almost undoubtedly the main avenue of infection in the part of the United States in which they were working.

In the same year, Kuenen and Swellengrebel (1913), in Java, carried on the first experiments on the carriage of cysts of intestinal amoebae by flies. They were also the first to use on cysts of intestinal protozoa the eosin test for viability (see section on methods), which has been used by all subsequent investigators. Their experiments with flies were not extensive. In one experiment, flies were much soiled with human feces containing cysts of *E. histolytica* and then examined for cysts. Three flies examined at once carried on their surface 25 living and 2 dead cysts, 41 living cysts, and 41 living and

3 dead cysts, respectively. Two more flies, examined one hour later, carried 27 and 52 cysts, all of which were dead. No cysts were found in the interior of any of the flies. In another experiment, flies allowed access for 48 hours to fecal material containing *E. histolytica* cysts showed no cysts, either externally or internally, when examined. Since they found no evidence of internal carriage, and had already found that cysts were killed in fifteen minutes or less by drying, these authors dismissed the carriage of cysts by flies as unimportant.

Thomson and Thomson (1916), in the course of an investigation into the intestinal protozoa of Egypt, noted that flies would greedily eat human feces containing protozoan cysts and that the cysts could be found in numbers in the alimentary tracts of the flies. They list the contamination of milk and drinking-water by flies among the methods by which cysts may be disseminated.

The most extensive piece of work on the carriage of cysts by flies is that of Wenyon and O'Connor (1917). Like Kuenen and Swellengrebel, they used the eosin test to determine whether cysts were living or dead. Their experimental results are summarized in Table I. The main points to be noted in these results may be listed as follows:

1. In most of their experiments, all the cysts found in the fly's intestine were recorded as living. In only two of their experiments was note made of the presence of dead cysts with the living ones.

2. The longest survival-time of cysts in the fly's intestine which they found was 24 hours for *Entamoeba histolytica* and *Giardia intestinalis,* 42 hours for *Entamoeba coli.*

3. Living cysts were deposited in the fly's feces for at least 16 hours after feeding, and motile forms of *Trichomonas* were found in the fly's feces 5 minutes after it had been fed on human feces containing these forms.

4. Cysts of *Entamoeba histolytica*, *Entamoeba coli* and *Giardia intestinalis* were found in the feces of flies caught wild.

In their own words, the conclusions from these experiments are given as follows (Wenyon and O'Connor, 1917, p. 145).

"(1) Flies feeding on feces readily take up encysted and other forms of protozoa into their intestine.

(2) The encysted forms of the protozoa can be found in the fly's intestine so long as any fecal matter remains there. If the flies are prevented from feeding this may be as long as forty-two hours.

(3) The cysts do not degenerate to any extent in the gut of the fly.

TABLE I.

Summary of the experimental results of Wenyon and O'Connor.

A. Flies fed on infected feces and dissected later.

Kind of cysts used.	Time between feeding and examination.	Number of flies whose intestines contained		
		Living cysts.	Living and dead cysts.	No cysts.
E. histolytica	18 hours	1	0	14
E. histolytica	20 hours	0	6	0
E. histolytica	24 hours	1	0	7
E. histolytica	42 hours	0	0	12
E. histolytica	70 hours	0	0	3
E. coli	24 hours	4	0	4
E. coli	42 hours	1	0	2
E. coli	70 hours	0	0	3
Giardia intestinalis	24 hours	0	5	3

B. Flies fed on infected feces and their own fecal droppings examined later.

Kind of protozoa used.	Time between feeding and examination.	Number of flies whose droppings		
		Contained living protozoa.	Were examined dry and dead cysts found.	Contained no cysts.
E. histolytica cysts	30 minutes	6	1	0
E. histolytica cysts	2 hours	4	0	0
E. histolytica cysts	5 hours	0	0	"small batch"
E. histolytica cysts	16 hours	6	0	0
E. coli cysts	40 minutes	6	0	0
Giardia intestinalis cysts	40 minutes	6	0	0
Giardia intestinalis cysts	5 hours	0	"small batch"	0
Trichomonas, motile	5 minutes	6	0	0

C. Examination of droppings of wild flies.

Total flies examined	229
E. histolytica cysts found in	5
E. coli cysts found in	6
Giardia intestinalis cysts in	1

(4) Flies can deposit in their own droppings material they have ingested only five minutes before."

From their conclusion (3) it is evident that Wenyon and O'Connor believe that protozoan cysts are not killed by a stay in the fly's intestine, as bacteria ultimately are. On the other hand, in two of their experiments both living and dead cysts were found in the fly's intestine. Wenyon and O'Connor used three species of flies, *Musca domestica*, *Calliphora* and *Lucilia*, the results being similar in all three.

Roubaud (1918) has also published a short paper on the rôle of flies as carriers of intestinal protozoa. This paper is in the nature of a summary and does not give the details of the actual experiments. He reports that:

1. Free amoebae (both *histolytica* and *coli*) fed to flies were found dead or moribund in the fly's intestine in less than an hour, never encysting.

2. Cysts of *Entamoeba coli, Entamoeba histolytica* and *Giardia intestinalis* traverse the digestive tract of the fly and are emitted, unaltered, with the fly's feces. They may be found in the feces for over 24 hours, but only rarely after 40 hours.

3. If a fly which has ingested cysts drowns in water, the cysts live for about a week, but are all dead by the time the fly decomposes enough to release them from its abdomen (this takes a month or more).

Roubaud used only *Musca domestica* in his experiments.

From the results of these various investigators, the only results so far available, it appears established that flies take up intestinal protozoa, both in the free and encysted state, from human feces; that the free forms die within an hour, but that the cysts persist in a living condition for 24 hours or more and are deposited, alive, in the fly's feces. These results also tend toward the view that the cysts are not harmed at all by their stay in the fly's intestine. This seemed remarkable, since the results in the case of intestinal bacteria are so different, and my own experiments were intended to test out this very point.

MATERIAL AND METHODS.

1. PROTOZOAN MATERIAL.—The protozoan material used has included free motile forms of *Entamoeba histolytica* and *Chilomastix mesnili,* and cysts of *Entamoeba histolytica, Entamoeba coli, Endolimax nana, Giardia intestinalis,* and *Chilomastix mesnili.* In experiments with the free forms and in a few of the cyst experiments, the flies were fed directly on a bit of fresh human feces containing the forms in question. In the majority of the cyst experiments, the cysts were concentrated and cleaned by a method devised by Dr. W. C. Boeck, now of the U. S. Public Health Service. This method involves emulsifying the stool in distilled water, straining the emulsion through several thicknesses of cheesecloth, throwing down the cysts and other solid particles by centrifugation and washing the sediment thus obtained (by repeated additions of distilled water, followed by cen-

trifuging or sedimentation and decantation of the supernatant liquid), until the water above the sediment appears clear to the naked eye. Material thus concentrated and washed will keep for several days or even weeks in a condition satisfactory for experimental work. Since this washed sediment has lost the odor which characterized the original fecal material, it was found advisable to add a bit of dilute syrup to this material before attempting to feed flies upon it.

I wish at this point to express my great indebtedness to Dr. W. C. Boeck for his kindness in furnishing me with cyst material and in discussing technique and results with me. My sincere thanks for assistance in securing material are also due to Dr. C. E. Simon and Dr. W. H. Taliaferro, of the School of Hygiene and Public Health, and to Dr. G. C. Payne of the International Health Board, now stationed in Trinidad, West Indies. Without the aid given by these gentlemen it would have been impossible for me to have carried out the series of experiments which I had planned.

2. Flies used.—The majority of my experiments were conducted with flies of the species *Musca domestica* (housefly) and *Calliphora erythrocephala* (blow fly) reared from larvae in the laboratory. A portion of the experiments were carried on with flies of other species, some caught wild and others reared in the laboratory. Other species used in small numbers were *Fannia canicularis* (lesser housefly), *Lucilia caesar* (green-bottle fly) and *Chrysomyia macellaria* (screw-worm fly). The results with all these species were similar. The larger green-bottles and blow flies ingested larger quantities of cyst material and retained it longer, and were therefore used for experiments involving a period of 40 hours or more between the time of feeding and time of examination.

3. Methods. (*a*) *Feeding flies.*—Flies were either used as soon as they had emerged from the puparium and become sufficiently hard to be handled, or were kept for several days in a large glass cylinder closed with netting and provided with a dish of syrup for food. In either case the flies to be used in an experiment were isolated in glass vials three quarters of an inch in diameter and two and a half inches long. When the flies had been transferred into these vials they were plugged with cotton and set aside for from 4 to 12 hours, so that the flies would be willing to feed readily. In feeding the flies, the cotton stopper was removed and the vial inverted over a glass slide on which had been placed a few drops of material containing the protozoa to be used. Ordinarily the fly discovered the food and

began to ingest it within a minute. Feeding continued for from one half to 3 minutes, depending on the size of the fly and the degree of starvation. When ingestion was finished the fly always left the food and walked up the side of the vial. At this point the cotton plug was again inserted and the vial set away until the fly was needed for dissection.

(*b*) *Examination of flies.*—Flies which had been fed on protozoan material were kept in the individual vials until examined. Where the time between feeding and examination exceeded 50 hours, the flies were fed once on sterile syrup about 30 to 36 hours after their feed on cysts. Even after this second feed, cysts could be found in the intestines of a small proportion of the flies. A fly which was to be examined was anaesthetized by putting a few drops of chloroform on the cotton plug of the vial. The fly was then removed from the vial, its legs and wings pulled off with forceps, and the body placed in a drop of normal salt solution on a slide. Under a binocular microscope the intestine was dissected out with spear-pointed needles and transferred to a drop of 1:10,000 eosin or neutral red solution on a second slide (Plate VI, Fig. 1). Still under the binocular, the intestine was carefully torn open and portions of the peritrophic membrane with the enclosed fecal masses were drawn out entire (Plate VI, Fig. 2). The remainder of the intestine was then removed and a cover glass pressed down on the drop containing the peritrophic membrane until the fecal masses were flattened out to about the thickness of a single cyst (Plate VI, Fig. 3). The fecal masses were then searched through with a compound microscope equipped with a mechanical stage. For this searching the 4 mm. objective and No. 10 ocular were used. The first fifty cysts encountered were taken as the sample and the number of these which remained unstained was recorded.

The use of a dilute solution of eosin as a test to determine whether a given protozoan cyst is living or dead was first recorded by Kuenen and Swellengrebel. It had been previously used by Strassburger and others in work on plant cells. Wenyon and O'Connor also used this test, concluding as a result of experiments designed to test its applicability that (Wenyon and O'Connor, 1917, p. 153):

> It seems, therefore, clear that the eosin-staining cysts are dead though it may be argued that others which do not stain may be dead also or, at any rate, non-infective. Still, if we accept the eosin test as a criterion and regard all unstained cysts as living, the error in judgment will be on the safe side.

Very early in my own work it was found that a similar solution of neutral red stained dead cysts in the same way as the eosin solution, and that in comparative experiments a slightly larger proportion of cysts were stained by neutral red than by eosin. Since its greater transparency makes the neutral red solution easier to work with and since it appears to be a slightly more delicate test than the use of eosin, all the experiments reported in this paper were carried out with neutral red. In the earlier work comparative series were also examined in eosin, but since they showed only a consistent decrease of from 2 to 4 per cent. in the number of cysts stained, they were not included in the tables.

A cyst which has stained with neutral red can be easily distinguished from an unstained cyst. Not only can the red color be seen inside the cyst wall, but there is also a decided loss in refractivity, and, in many cysts, a decided plasmolysis and degeneration of the cyst contents. In cysts which have taken the stain only lightly, the most prominent differences are the lower refractivity and the appearance of the nuclei, which are usually readily visible in stained cysts but invisible or barely to be made out in unstained ones.

(*c*) *Numerical Expression of Results.*—In all previous work it has been assumed, seemingly, that the cysts all died at the same time, and as I have already brought out in my account of Wenyon and O'Connor's work, only twice did they record the finding of both stained and unstained cysts in their flies. My first series of experiments showed that this was not the case. During the time the cysts remain in the fly's intestine there is a gradual increase in the proportion of stained cysts until finally all are stained. In order to reduce this to a numerical expression I adopted the plan of counting the first fifty cysts seen in any preparation and recording the number of these cysts which remained unstained, which were presumably alive. Thus, throughout my tables, the numbers set below the various time intervals represent the number of living (unstained) cysts found in fifty cysts examined. Multiplying this number by two will in every case give the percentage of cysts still alive after this time interval. The number given under the heading "Original Material" represents in each case the number of unstained cysts found out of fifty cysts counted in a sample of the material fed to the flies, taken just before feeding began.

Results.

1. *Survival of free forms of Entamoeba histolytica and Chilomastix mesnili in the fly's intestine.*

I have carried on only two experiments with free, motile forms. I include them here to confirm the similar observations of Roubaud on *Entamoeba histolytica* and of Wenyon and O'Connor on *Trichomonas.*

(*a*) *Entamoeba histolytica.*—Dec. 2, 1919. Five flies fed on drops of mucus taken from a dysenteric stool containing large numbers of motile amoebae with included red blood corpuscles.

Fly No. 1—examined after 7 minutes—amoebae unstained, some moving feebly.
Fly No. 2—examined after 20 minutes—none moving, most of them stained.
Fly No. 3—examined after 30 minutes—none motile, all stained.
Fly No. 4—examined after 1 hour—none motile, all stained.
Fly No. 5—examined after 1 hour—none motile, all stained.

This corroborates Roubaud's statement that free amoebae swallowed by flies die in less than an hour, never encysting.

(*b*) *Chilomastix mesnili.*—Nov. 8, 1919. Five flies fed on drops of a diarrheic stool containing large numbers of motile *Chilomastix.*

Fly No. 1—examined after 7 minutes—motile *Chilomastix* numerous, both in intestinal contents and in feces squeezed out of rectum.
Fly No. 2—examined after 20 minutes—a few specimens moving feebly, most of them non-motile and stained.
Fly No. 3—examined after 1 hour—all non-motile and stained.
Fly No. 4—examined after 1 hour—all non-motile and stained.
Fly No. 5—examined after 1 hour—all non-motile and stained.

This confirms Wenyon and O'Connor's findings with the closely related flagellate *Trichomonas*, showing that although the motile flagellates are killed in less than an hour, they may pass through the digestive tract and appear in the fly's feces in a few minutes.

2. *Survival of cysts of intestinal protozoa in the fly's intestine.*

(*a*) *Entamoeba histolytica.*—My experiments on the survival of the cysts of *Entamoeba histolytica* were all carried out on cysts from a single stool. Cases of amoebic dysentery are not common in Baltimore, and among the half dozen or so from which I was able to obtain specimens during the course of this work, only a single one proved to be a source of cysts. This stool was washed and concentrated on Dec. 9, 1919, and flies were fed on Dec. 9 (48 cysts out of 50 unstained), Dec. 10 (45 cysts out of 50 unstained) and Dec. 15–17 (37 cysts out of 50 unstained). The results of examinations for cysts are summarized in Table II. It will be observed that while no single series is complete, the different series supplement each other to such an extent that the composite record is reasonably complete, except for the gap between 24 and 42 hours. Although incomplete to this extent, the records show the same characteristics which appeared in all my experiments on the survival of cysts, and which may be summarized as follows:

TABLE II.

Survival of cysts of Entamoeba histolytica in the fly's intestine.

Original material.	Number of hours between feeding and examination.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
48......	48	48 48																		20
45......																	22	23		
37......							34	33		30	31	28	27 26		23 24				17	

Original material.	Number of hours between feeding and examination.																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
48......	16			14 11																
45......		18																		
37......	14			13																

Original material.	Number of hours between feeding and examination.																			
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
48......																				
45......		6						2												
37......								2	1 1					0				0		

1. For a short time (2 hours in this case) no deaths occur, the number of cysts which stain being no greater than in the original material.

2. Next, for a time, there is a high death rate. The number of cysts which stain increases rapidly until (in 15 hours in this case) more than half of the cysts found are stained.

3. After this the cysts which still remain unstained die off more slowly, until finally all are found to be stained.

The results of the experiments on the survival of cysts of *En-*

tamoeba histolytica in the fly may be summarized in a single sentence: The experiments were carried out with cysts from a single source; cysts were found in 29 flies dissected at varying times after feeding; no deaths occurred for 2 hours; half the cysts were dead after 15 hours and the last living cysts found were in two flies examined 49 hours after they had fed on fecal material containing the cysts.

(*b*) *Entamoeba coli.*—Cyst carriers of *Entamoeba coli* are more numerous in Baltimore than carriers of *Entamoeba histolytica*, but for some reason, possibly because of the large size of the cysts, it was difficult to carry on experiments with the cysts of the former. Several times a large number of flies were fed on cyst material, but although their intestines contained abundant fecal material when examined, no cysts were found. It was finally discovered that when freshly emerged flies, either houseflies or blue-bottles, were given their first feed on cyst material, the cysts were ingested in large numbers. The results summarized in Table III were obtained in this

TABLE III.

Survival of cysts of Entamoeba coli in the fly's intestine.

Original material.	Number of hours between feeding and examination.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
46......		46	45	44		42	41									20				22
45......		45		40		42		40		30		30		25		20	22	19		18
		44																		
40......	40	40		37		31										29				25
		40		36												30		26		22
		39		36												29		25		23
				38												28				
																28				

Original material.	Number of hours between feeding and examination.																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
46......		19																		
45......		15		15		14		11		10		9		6						6
				14		12		11		9										
40......		22		19		15		11		9										
		20		19																
		20		17																

Original material.	Number of hours between feeding and examination.																			
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
46......								4							0					
45......		5		3		4														
40......		6		4		3		2		1		1					0			
						2						0								
						2														

way. The first successful experiments were carried out on Feb. 25, 1920 on material obtained in Baltimore (46 cysts out of 50 unstained). Then pressure of teaching duties delayed the resumption of experiments until June 7, 1920, when a second series was completed, also on Baltimore material (45 cysts out of 50 unstained). The remainder of this material was accidentally destroyed and the final series was carried out on July 6, 1920 on washed and concentrated material sent from Trinidad by Dr. G. C. Payne (40 cysts out of 50 unstained).

As can be readily seen from Table III, I was able to obtain much more complete series in the case of *Entamoeba coli* than in that of *Entamoeba histolytica.* Each of the three series is fairly complete, and in the composite series the largest gap is only six hours, from 34 to 40 hours after feeding. This series shows the same characteristics as in the case of *Entamoeba histolytica* and, as was to be expected from the close relationship between them and the similarity of their cysts, the results are almost identical with those obtained in working with the pathogenic species.

To summarize the work on *Entamoeba coli,* the experiments were carried out with cysts from three different sources; cysts were found in 75 flies dissected at varying time intervals after feeding; deaths were just beginning to occur at the end of two hours, half the cysts were dead in 14, 16 and 18 hours in the three different series; and the last living cyst was found in a fly of the third series, dissected 52 hours after feeding.

(*c*) *Endolimax nana.*—Cysts of *Endolimax nana* were not available at a time when I was able to carry on experiments with them until my work with the other species was completed. I can report only a single series of experiments with these cysts, the flies being fed on October 12 and 16 hours, the other between 32 and 37 hours. Since the cysts out of 50 unstained), furnished and identified by Dr. W. C. Boeck. The series is almost complete, there being only two gaps, one between 12 and 16 hours, the other between 32 and 37 hours. Since the cysts of *Endolimax nana* closely resemble those of the other intestinal amoebae, we would expect the time of survival in the fly's intestine to be about the same. The single series of experiments summarized in Table IV agrees closely with those carried out on the other amoebae, except that the last few cysts died off more rapidly than in the case of *Entamoeba histolytica* or *Entamoeba coli*, the last living cyst found occurring at 39 hours instead of somewhere around 50 hours. Since Dobell has found morphological characters which he considers justify the separation of *Endolimax nana* as the type of a new genus, *Endolimax,* it may be that this difference in survival time is significant. My scanty data do not, however, allow me to more than suggest this as a possibility.

TABLE IV.

Survival of cysts of Endolimax nana in the fly's intestine.

Original material.	Number of hours between feeding and examination.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
47......		47		45		41		36		34		31				27		25	25	21 18

Original material.	Number of hours between feeding and examination.																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
47......	17	18		15		11		7		6		5					3		1	

Original material.	Number of hours between feeding and examination.																			
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
47......	0		0																	

To summarize, the experiments on the survival of the cysts of *Endolimax nana* were all carried out with cysts from a single source; cysts were found in 22 flies dissected; no deaths occurred for 2 hours, half the cysts were dead in 18 or 19 hours, and the last living cyst was found 39 hours after the fly was fed.

(*d*) *Giardia intestinalis.*—Cases in which infections with *Giardia* (*Lamblia*) *intestinalis* occur in children are not uncommon in Baltimore, and it was often possible to obtain good cyst material. These cysts are much less resistant to unfavorable conditions of any kind than those of the amoebae, and experiments had to be carried out as soon as the material came in, in order to have any considerable percentage of the cysts alive at the beginning of the experiments. The results summarized in Table V were obtained as follows: October 30, 1919, material from Baltimore (40 cysts out of 50 unstained); November 15–17, 1919, Baltimore material (40 cysts out of 50 unstained); November 18, 1919, same material as the last (31 cysts out of 50 unstained; November 21, 1919, same material as the last (27 cysts out of 50 unstained); November 5, 1920, material from Washington, D. C. (40 cysts out of 50 unstained).

TABLE V.

Survival of cysts of Giardia intestinalis in the fly's intestine.

Original material.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
	Number of hours between feeding and examination.																					
40....	40			38 39 35	34 33	29 31 37	29	18	14 20	11 16	8	15 6	12 2		2	1				0	0 0 0	
31....	31	30																	0 0	0 0	0	0
27....		25							17	12	4	1										

As can be seen from the table, the cysts of *Giardia* succumb to the unfavorable influences in the intestine of the fly much more rapidly than do the cysts of the amoebae. Because of this high death rate throughout the stay of the cysts in the intestine of the fly, it is more difficult here to demonstrate that the last half of the cysts die more slowly than the first half. By making a rough interpolation, it can be said that 50 per cent. of the cysts die in seven and a half hours, while it takes eight and a half hours more before the remaining 50 per cent. are all dead, but the difference is not as evident as in the case of the other cysts.

Summarizing as before, cysts of *Giardia intestinalis* from three different sources were used in the experiments; cysts were found in 40 of the flies dissected; no deaths occurred for 1 hour, half the cysts were dead after 8 hours, and the last living cyst encountered was found in a fly dissected 16 hours after feeding.

(*c*) *Chilomastix mesnili.*—Through the kindness of Dr. C. E. Simon, Dr. Boeck and I were introduced to a gentleman with a chronic diarrhea accompanied by a severe infection with *Chilomastix*. Although we obtained stools from this case twice a week for several months, only on one occasion were cysts at all numerous. The series of experiments in Table VI were all carried out on this sample, the flies being fed on January 30 and February 1, 1920, at which time no stained cysts were found among the fifty examined. As the table shows, the cysts of *Chilomastix* are by far the most resistant of any with which I have carried on experiments, a few of them still being found unstained after 80 hours in the fly's intestine. The necessity of feeding the flies on sterile syrup during this period, in order to keep them alive, and the small percentage of flies in which cysts could be found after this feeding, made it impossible to obtain more frequent records in the latter part of the series. Here, even when starting with material in which no dead cysts were to be found, we see clearly that the additional time required to kill the last half of the cysts is greater than the time required to kill the first half. Half of the cysts, in this case, stained up at the end of 36 hours, but at the end of 44 hours more, two cysts out of fifty were still unstained.

TABLE VI.

Survival of cysts of Chilomastix mesnili in the fly's intestine.

Original material.	Number of hours between feeding and examination.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
50......		50		47		43		40		37		36		34		33		32		31

Original material.	Number of hours between feeding and examination.																			
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
50......		29		28								26				25		24		

Original material.	Number of hours between feeding and examination.																			
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
50......								19						16						

Original material.	Number of hours between feeding and examination.																			
	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
50......		12						10				7								2

Original material.	Number of hours between feeding and examination.									
	81	82	83	84	85	86	87	88	89	90
50..........						0				

To summarize: In the experiments with cysts of *Chilomastix,* the cysts came from a single source; cysts were found in 22 of the flies dissected; no cysts died for at least 2 hours, half of the cysts were dead after 36 hours, and the last living cysts found had been in the fly's gut for 80 hours.

3. *Survival of cysts in drowned flies.*

In order to test Roubaud's report that cysts of intestinal amoebae remained alive for a week or more in drowned flies, a single series of experiments were carried out with cysts of *Giardia intestinalis* and *Entamoeba histolytica,* which were fed to houseflies and bluebottles, and the flies then at once drowned in a vial of water. The results are given in Table VII. The results corroborate Roubaud's findings, some

TABLE VII.

Survival of cysts of Entamoeba histolytica and Giardia intestinalis in drowned flies

Species of cysts.	*Giardia intestinalis.*		*Entamoeba histolytica.*	
Species of fly.	*Calliphora erythrocephala.*	*Musca domestica.*	*Calliphora erythrocephala.*	*Musca domestica.*
Original material	30	30	45	45
Number of cysts remaining unstained after—				
1 day	26	26	40	38
2 days	24	20	33	34
3 days	12	16	26	24
4 days	6	8	19	20
5 days	0	0	11	13
6 days	0	0	5	4
7 days			2	1
8 days			0	0

of the amoeba cysts being still unstained after 7 days. I have already shown that the cysts of *Giardia* are less resistant than the amoeba cysts, so it is not surprising to find that the last living *Giardia* cysts were found on the 4th day. In this, as in all my other experiments, no difference was found between the behavior of cysts in different species of flies.

4. *Notes on feeding in flies, etc.*

I wish to treat briefly under this heading some points of interest that have come up during the course of this work, but which do not, strictly speaking, form a part of it.

(*a*) *How much food can a fly ingest at one time?*—Wenyon and O'Connor (1917, p. 143) attempted to determine how much fecal material a fly would take up during half an hour, by weighing small quantities of feces on a coverglass, exposing them for half an hour to some flies which had been starved for 2 or 3 hours, and then weighing them again, a deduction being made for evaporation on the basis of the loss in weight during the same time by a control preparation exposed under the same conditions except for the presence of the flies. Their results varied from 0.0005 to 0.0027 grammes per fly, averaging about 0.001 gm. per fly per half hour. This seemed to me a very small quantity, so I attempted to approach the problem in a somewhat different way.

A number of flies were starved over night (*i.e.*, from 17 to 21 hours) in individual vials. In the morning they were transferred into a single vial and weighed. Then they were transferred back into individual vials, fed individually on small separate drops of dilute syrup, transferred into the same single vial and weighed again. The weight of the syrup eaten was obtained by subtraction, and since the weight of a known volume of the syrup was easy to obtain, the volume as well as the weight of the food eaten could be calculated.

Two possible causes of error in this method are the soiling of the fly's surface with the food, and the deposition of fecal droplets in the individual vials before the flies were transferred into the vial in which they were weighed. As to the first, Graham-Smith (1910, p. 5) has already pointed out that flies fed on small separate drops of food-substances soil themselves very little, and I have also found this to be the case. Further, the ten to twenty minutes during which the flies which had been fed remained in their individual vials before they were transferred to the weighing vial, allowed a considerable time for cleaning. As to the second point, flies starved for as long a period as over night have usually emptied their intestines, and my experience has been that they do not ordinarily begin depositing fecal droplets for nearly an hour after they have fed. The results of these experiments were as follows:

House fly—*Musca domestica.*

1. 8 flies—starved 17 hours—gain in weight after feeding, 0.031 gm. or 0.004 gm. per fly; this corresponds to a volume of 0.0039 c.c.
2. 9 flies—starved 21 hours—gain in weight after feeding, 0.063 gm. or 0.007 gm. per fly; this corresponds to a volume of 0.0066 c.c.

Blue-bottle fly—*Calliphora erythrocephala.*

3. 5 flies—starved 17 hours—gain in weight after feeding, 0.117 gm. or 0.023 gm. per fly; this corresponds to a volume of 0.022 c.c.

Taken in conjunction with the results of Wenyon and O'Connor, these results point to the conclusion that the amount of food eaten by a fly at a single meal depends very largely on the length of time that it has been without food previously. Also, the larger amounts of food taken in by larger flies, like the bluebottles, must tend to increase their opportunities for carrying protozoan cysts and bacteria, and thus, to a certain extent, counteract their more infrequent occurrence in houses.

(*b*) *How soon after a fly has fed do cysts appear in the feces, and* *how long after feeding can they be found in the feces?*—All the work on dissection of flies which have been fed on cysts bears on this point, and I have also carried on some experiments in which flies were fed on dead yeast cells stained with neutral red. From these experiments I have concluded that the length of time which elapses between feeding and the deposition of feces depends very largely on the length of time since the flies have previously fed. A fly which has been starved for only an hour or so, may deposit feces immediately after feeding, and these feces may contain material that has just been eaten, as shown by Wenyon and O'Connor's observations on motile *Trichomonas,* and my own on motile *Chilomastix.* On the other hand, in a number of experiments where flies starved over night were fed on stained yeast cells, no fecal droplets were usually deposited for an hour after feeding, and the earliest fecal deposit noted in the whole series was 47 minutes after the conclusion of the feed.

In regard to the length of time during which cysts may be found in the feces, my experiments with stained yeast cells showed that if a fly is not fed again, the stained cells may be recognized in the feces up to the time of death, say about 48 hours, on the average. As I have already noted in the work on the survival of cysts, cysts can sometimes be found in the intestinal contents, and therefore undoubtedly in the feces, up to 86 hours after the infective feed, provided the fly is kept alive by feeding it on syrup in the meantime. Such cases are probably due to retention of some of the original feed in the crop of the fly, even after the intestines are practically empty. Graham-Smith (1914, p. 73) has noted this very phenomenon in flies fed on suspended carmine granules.

(*c*) *Notes on the occurrences of Herpetomonas in Baltimore flies.*—During my various experiments, I have dissected considerable numbers of flies and studied their intestinal contents carefully enough, so that a herpetomonad infection would almost assuredly have been noticed whenever it existed. It may be worth while to add the data on this point, for comparison with similar records from other parts of the world. My records include the dissection of 396 flies of the following species:

Calliphora erythrocephala	124
Musca domestica	225
Lucilia caesar	25
Chrysomyia macellaria	19
Fannia canicularis	3

TABLE VIII.

Survival of different protozoan cysts in the fly's intestine; averages compiled from Tables II to VI, inclusive.

Species.	Number of hours between feeding and examination.																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Giardia intestinalis	35.5	27.5		37.1	33.5	32.3	29	18	17	13	6	7.3	7		2	1			0	0	0	0						
Endolimax nana		47		45		41		36		34		31				27		25	25	19.5	17	18		15		11		7
Entamoeba histolytica	48	48					34	33		30	31	28	26.5		23.5		22	23	17	20	15	18		12.6				
Entamoeba coli	40	42.3	45	38.5		38.1	41	40		30		30		25		26.3	22	23.3		22		19.2		16.8		13.6		11
Chilomastix mesnili		50		47		43		40		37		36		34		33		32		31		29		28				

Species.	Number of hours between feeding and examination.																										
	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
Giardia intestinalis																												
Endolimax nana		6		5					3		1		0		0													
Entamoeba histolytica														6						2	1					0		
Entamoeba coli		9.1		9		6						6		5.5		3.5		3.6		3		1		0.5			0	
Chilomastix mesnili				26				25		24										19						16		

Species.	Number of hours between feeding and examination.																						
	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
Entamoeba histolytica		0																						
Entamoeba coli	0																							
Chilomastix mesnili						12						10				7								2

I have found only three cases of herpetomonad infection, one in *Musca domestica* (probably *H. muscae-domesticae*) and two in *Calliphora erythrocephala* (a larger species, probably *H. calliphorae*).

DISCUSSION.

In order to be able to compare the times of survival of the different cysts within the fly's gut, I have averaged the results of the different series carried out on each species of cyst and summarized them in Table VIII. To permit of easier comparison, the graphs in Fig. 4 (Plate VII) were constructed from the data in this table. The numerous irregularities in the early part of the curves are largely due to the throwing together of different series, as can be seen, for example, by comparing the data on *Giardia intestinalis* in Table VIII with Table V, where the series are shown separately. The proportion of living cysts in the original material has a decided influence on the early portion of the curve, but appears to be less important in the last half of it.

As is plainly brought out in Fig. 4, the cysts studied fall into three distinct groups. The cyst of *Giardia intestinalis* is by far the least resistant, that of *Chilomastix mesnili* the most resistant. Intermediate between these two we find the amoeba cysts, *Entamoeba coli* and *histolytica* and *Endolimax nana*. The curves for all three species practically coincide up to about 25 hours, after which time the curve for *Endolimax nana* drops more steeply than those of *Entamoeba histolytica* and *coli*, ending at about 40 hours, as compared with about 50 hours for the other two species. Since my observations on the cysts of *Endolimax nana* were confined to a single series, this difference cannot be affirmed to have any significance. In view of the fact that the genus *Endolimax* has been separated from *Entamoeba* this apparent difference in the viability of the cysts is suggestive, but no more. Further work is needed to determine this point.

It is of great interest to note that Dr. W. C. Boeck, while working in this laboratory on the resistance of cysts to heat, secured results of the same general trend as my own. Dr. Boeck's work is soon to appear in this journal, but he permits me to state here that he finds that the cysts of *Giardia* die at a lower temperature than *histolytica* cysts, and these, again, at a lower temperature than the cysts of *Chilomastix*.

It seems probable, then, that the degree of resistance of cysts to various kinds of external influences is determined by some common factor, and it is at least a tempting hypothesis to suggest that this factor is the thickness of the cyst wall. While I have not attempted to measure the diameter of the cyst wall in any of these forms, a careful study of the living cysts certainly gives one the impression that the cyst walls of *Giardia* are thinner and the cyst walls of *Chilomastix* decidedly thicker than those of the amoeba cysts.

Turning to the practical aspects of the work, it is hoped that these results may be of interest to men working in the field on the general subject of soil pollution, first, because they emphasize again the importance of flies as carriers of disease-producing organisms from human feces to human food, and second, because they define more exactly the limits of such carriage, in so far as the cysts of intestinal protozoa are concerned. From this point of view, the relation of flies to the spread of intestinal protozoan infections may be stated as follows:

Flies feeding on a human stool containing cysts or free forms of intestinal protozoa will take large numbers of them into their intestines and deposit them again in their own feces. Since all stages of the protozoa are killed within a few minutes by drying, such fly feces are only dangerous to human beings when deposited on moist or liquid foods. A fly which has once ingested fecal material containing protozoa may deposit feces of its own which contain the infective forms of the protozoa at any time, from a few minutes after feeding until the most resistant forms, the cysts, are dead. The experiments reported in this paper show that the time of survival varies for the cysts of different species of protozoa and that the extreme limits are about as follows:

Free forms	1 hour or less.
Cysts of *Giardia*	16 hours.
Cysts of amoebae	40 to 50 hours.
Cysts of *Chilomastix*	80 hours.

These experiments also show that half of the cysts contained in the fly will be alive 7 hours after feeding in the case of *Giardia*, 14–16 hours after feeding for the amoebae and 36 hours after feeding for *Chilomastix*. The earlier hours, when a large proportion of the cysts are still living, must be far more dangerous than later hours.

Further, if a fly containing cysts is drowned in water, milk, soup or other liquid food, the cysts will live still longer, about a week.

and there seems to be a possibility that human beings might be infected by swallowing such drowned flies.

SUMMARY.

1. Free forms of *Entamoeba histolytica* and *Chilomastix mesnili* ingested by flies were killed in an hour or less, not encysting. Motile *Chilomastix* were observed in a fly's feces 7 minutes after it had fed on a stool containing them.

2. Cysts of the intestinal protozoa ingested by flies were gradually killed in times varying with the species:

Entamoeba histolytica—half the cysts dead after 15 hours, last living cysts observed after 49 hours.
Entamoeba coli—half the cysts dead after 14 hours, last living cysts observed after 52 hours.
Endolimax nana—half the cysts dead after 18 hours, last living cysts observed after 39 hours.
Giardia intestinalis—half the cysts dead after 8 hours, last living cysts observed after 16 hours.
Chilomastix mesnili—half the cysts dead after 36 hours, last living cysts observed after 80 hours.

3. When flies which had ingested cysts were drowned in water, the cysts survived much longer:

Giardia intestinalis—half the cysts dead after 2 days, last living cyst observed after 4 days.
Entamoeba histolytica—half the cysts dead after 3 days, last living cyst observed after 7 days.

4. In experiments designed to test the maximum capacity of flies at a single meal, it was found that a single housefly (*Musca domestica*) could ingest 0.0068 c.c. of fluid and a single blow fly (*Calliphora erythrocephala*) could ingest 0.022 c.c.

5. An infection with *Herpetomonas muscae-domesticae* was found in only one out of 225 specimens of *Musca domestica* examined at Baltimore. Two infections with *Herpetomonas calliphorae* were found in 124 specimens of *Calliphora erythrocephala* examined.

REFERENCES.

GRAHAM-SMITH.
1910. Observations on the ways in which artificially-infected flies (Musca domestica) carry and distribute pathogenic and other bacteria. Reports to the Local Govt. Board on Public Health and Medical Subjects. New Series No. 40, pp. 1–41.

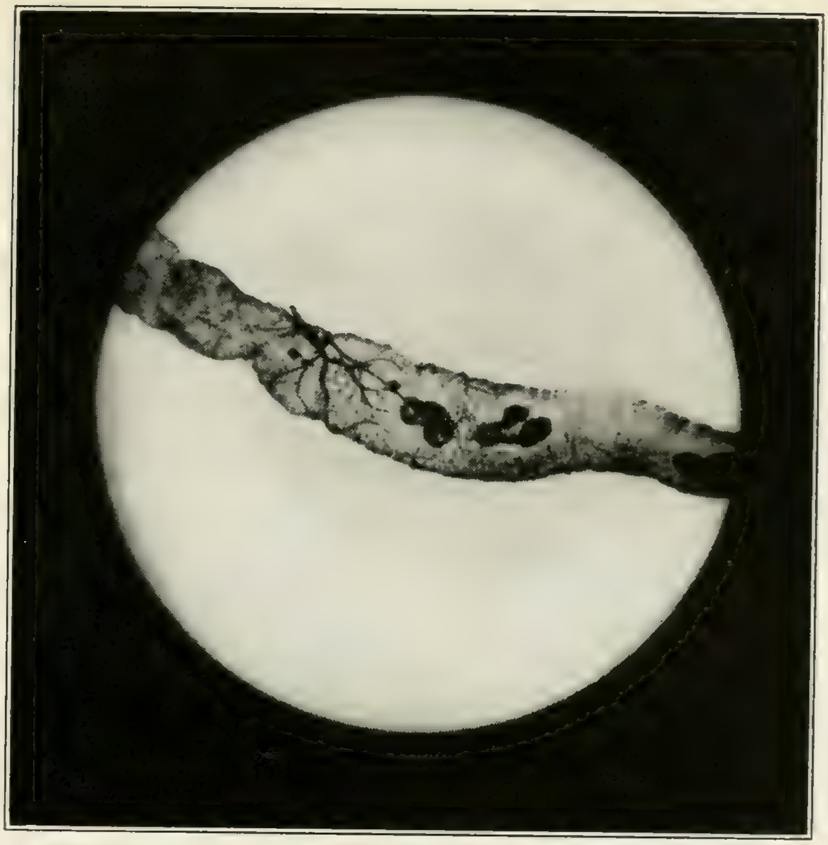

Fig. 1.

Fig. 2.

PLATE VI.

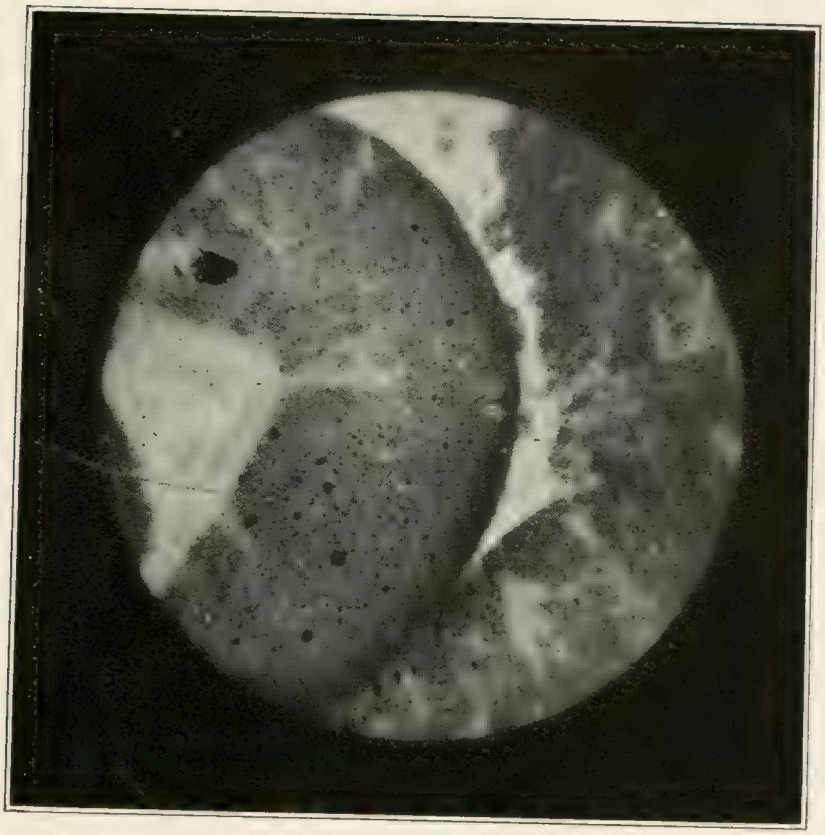

Fig. 3.

PLATE VII.

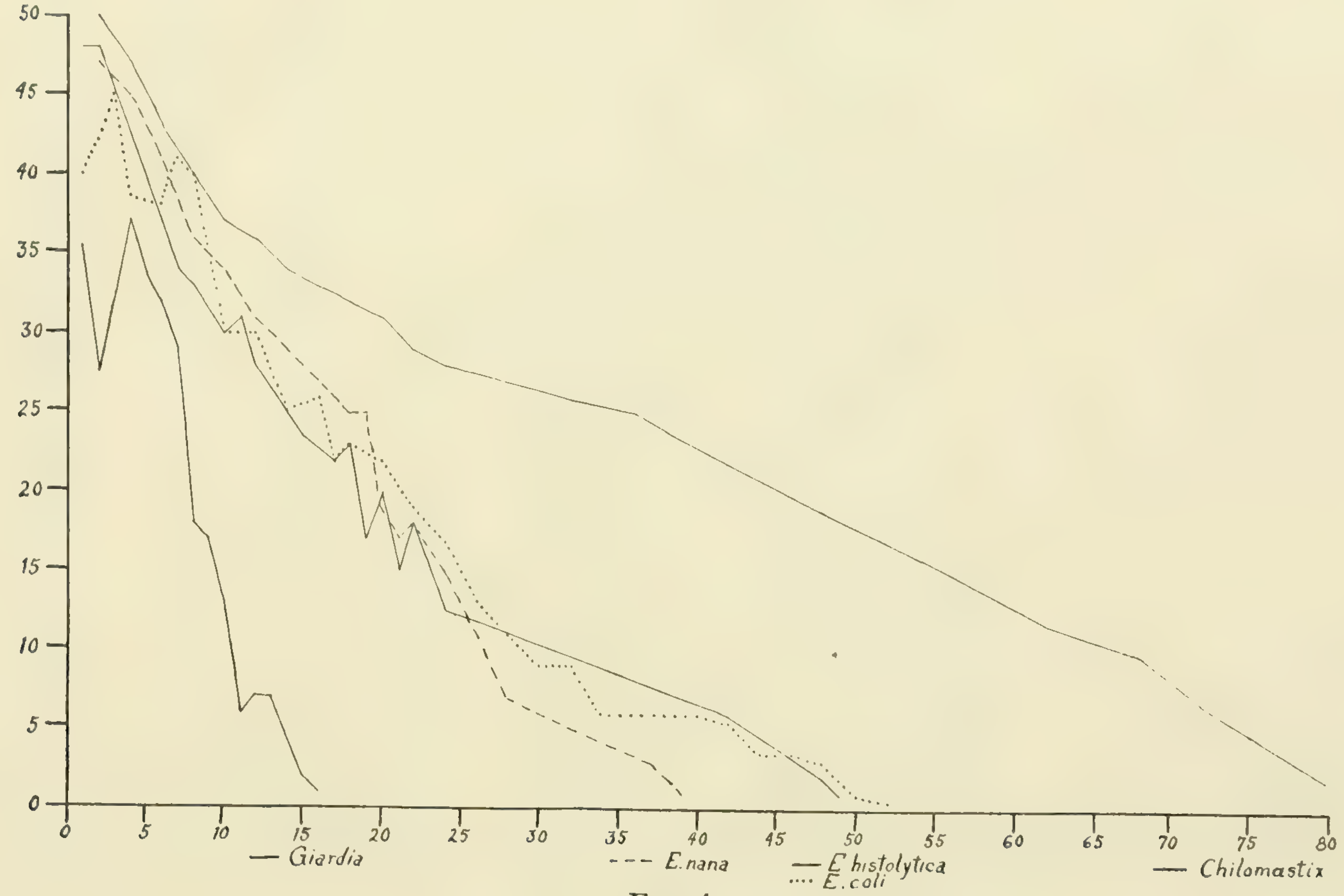

Fig. 4.

PLATE VIII.

1914. Flies and Disease. Non-bloodsucking Flies. Cambridge Univ. Press. 389 pp.

KUENEN AND SWELLENGREBEL.

1913. Die Entamöben des Menschen. Centralblatt für Bakt. und Parasit. I Abt. Orig., Bd. LXXI, pp. 378–410.

ROUBAUD.

1918. Le rôle des mouches dans la dispersion des amibes dysentériques et autres protozoaires intestinaux. Bull. Soc. Path. Exot., XI, pp. 166–171.

STILES AND KEISTER.

1913. Flies as carriers of Lamblia spores. Public Health Reports, XXVIII, No. 48, pp. 3–7.

THOMSON AND THOMSON.

1916. Protozoological Researches, including investigations on the sand in Egypt. Journ. Roy. Army Med. Corps, XXVII, No. 1, p. 1.

WENYON AND O'CONNOR.

1917. Human Intestinal Protozoa in the Near East. Wellcome Bureau of Scientific Research, London. 218 pp.

EXPLANATION OF PLATES.

FIG. 1. Photomicrograph of a small portion of a fly's intestine. The dark bodies showing through the intestinal wall are fecal masses.

FIG. 2. Photomicrograph of a loop of the peritrophic membrane, containing a number of fecal masses, which has been drawn out through a tear in the intestinal wall.

FIG. 3. Photomicrograph taken with much higher magnification than Figures 1 and 2, showing a portion of a single fecal mass, flattened out under a cover slip, but still enclosed within the peritrophic membrane. The scattered, oval, transparent bodies are cysts of *Giardia intestinalis* ingested fifteen hours previously.

FIG. 4. Graphs showing the gradual death of different protozoan cysts in the fly's intestine. Data taken from Table VIII. Ordinates show the number of cysts unstained out of fifty cysts examined, abscissae show number of hours between feeding and examination.

Observations on Abnormal Courses of Infection of *Paragonimus Ringeri*

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OBSERVATIONS ON ABNORMAL COURSES OF INFECTION OF *PARAGONIMUS RINGERI* *

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It is a well known fact that infection with *Paragonimus ringeri* is caused by the swallowing of the encysted larvae. It is, however, important to know whether these larvae, if freed from their cysts, can infect through the skin, mucous membrane or any wound on the skin of the host. It seems that this might be possible since they can penetrate the intestinal wall, diaphragm, connective tissues, muscles and some of the viscera of the host after the encysted larvae are swallowed. To test the possibility of such infections, we carried through experiments in an attempt to answer the following questions.

1. Can the freed larvae penetrate the sound skin of the host? The larvae after being freed from their cysts are injured in fresh water, and lose their power of movement, so that some other medium was necessary for the experiments. They are more active in artificial intestinal juice or in normal saline. To determine whether active larvae can penetrate the skin of the host under suitable conditions we experimented on mice, cats and new-born puppies. The results of these experiments are as follows:

(a) Although the freed larvae move actively in the artificial intestinal juice or normal saline, yet they can not penetrate the sound skin of the mice and puppies in a room temperature below 30° C., since their movement decrease below 37° C.

(b) We stretched three mice on a small plate after shaving off the hair of the abdominal wall and dropped upon them the artificial intestinal juice containing the freed larvae. We then put them into a warm chamber at 38° C. After one and a half hours we found that in one case there was a slight desquamation on the abdominal wall, but we could find no evidence of penetration by the larvae.

These experiments prove that the freed larvae cannot penetrate through the sound skin of the host.

2. Can the freed larvae infect through a wound in the body? It is conceivable that the freed larvae might penetrate a wound on the body. It is necessary, however, to try by experiment whether they can actually infect from an exposed wound of the host. On July 15, 1918, we dropped the normal saline with many freed larvae on fresh wounds, which we made in the backs of two dogs. After awhile we found

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several points of hemorrhages caused by the perforation of these larvae. Then we covered the wound with a watch glass and bound it to the body in order to prevent infection from the licking of the wound. One of the dogs died on August 20, thirty-five days after the experiment, and the other on September 10, having passed fifty-seven days after the experiment. By dissection, we could not find any distomes in the first dog, but in the last case two freed distomes were discovered in the chest cavity.

By these experiments we proved that occasionally the freed larvae can infect a host from a fresh wound on the body.

3. Can the freed larvae infect the host through the mouth? In Korea, R. Kawamura *(Tokyoer med. Woch. No.* 1986, 1916*)* proved experimentally that *Paragonimus ringeri* which is growing in its final host, can continue development after being fed to other animals. This mode of infection is very interesting in relation to lung distome disease, because many Koreans have the habit of eating the meat and the liver of dogs and other animals raw. If such animals had the young distomes wandering in their muscles, connective tissues and liver, this habit might lead to the infection of man. Therefore, we examined this point very carefully, using seven dogs, with these results:

(a) A new-born puppy was fed with 25 larvae, which were just freed from the cysts in the artificial intestinal juice, on July 31, 1917. It was killed on October 2, sixty-one days after feeding. On dissection, we found a wormcyst in the middle lobe of the left lung, in which were two distomes, and a freed distome in the left pleural cavity.

(b) A new-born puppy was fed with 25 larvae which had just been freed from their cysts, on Aug. 2, 1917. The puppy died on August 23, having passed twenty-one days after feeding. On dissection the next day, we found a distome in its right chest cavity, and numerous ankylostomes and ascarids which caused the death of the dog.

(c) A young dog was fed on Dec. 9, 1916, with 15 distomes which had lived for forty-two days in another dog, and killed on the twenty-fifth of the same month, fourteen days after feeding. On dissection we could not find any worms in the body.

(d) A young dog was fed on Aug. 19, 1918, with 20 distomes, which had lived for forty-two days in another dog. The dog died on the thirtieth day of that month, ten days after feeding. On dissection, we could not find any distomes in its body.

(e) A young dog was fed on July 8, 1918, with 27 distomes, which had lived for fourteen days in another dog, and 51 distomes on the thirty-first of the same month, which had lived for twenty days in another dog. Next day we killed this dog, five days having passed after the first and one day after the second feeding. On dissection, an hemorrhage was observed in the intestinal wall. This hemorrhage probably was caused by the action of the distomes, therefore we looked for the worms with special care, but could not find any worms in the body.

(f) A young dog was fed on June 15, 1918, with 18 distomes, which had lived for eighteen days in another dog, and on the nineteenth of that month with 9 distomes which had lived for twenty-three days in another dog. On June 21 it was killed, having passed six and two days after first and second feeding. Careful dissection failed to disclose any worms.

(g) A young dog was fed on June 10, 1918, with 228 larvae just freed from their cysts and killed twenty hours after feeding. On dissection, some inflammation was observed here and there on the serous membrane of the viscera, and many hemorrhages were found in the intestinal wall. It is evident that these hemorrhages were caused by the perforation of the distomes, because we found 21 distomes in the abdominal cavity. The diaphragm and the lungs were intact. There were no distomes in the pleural cavity.

From these experiments we learned that the larvae which were just freed from the cysts as well as the encysted larvae can infect by way of the mouth, but that distomes which were in a more advanced stage of development in their host, cannot develop after feeding to other animals. This indicates that the worms, which are partly developed in one host, find it difficult to pierce the wall of the intestine when introduced into another host, because of the decrease in activity which comes with growth. We proved this fact by using the mucous membrane of lips and the conjunctiva of dogs. For example, if we put some newly freed larvae on the conjunctiva, or on the mucous membrane of the lips of a dog, they soon penetrate the mucous membrane, but the distomes, which are partly developed in one host, cannot bore through these membranes. While our experiments showed that partly grown larvae of *Paragonimus ringeri* could not be transfered from one host to another, two cases of such infection have been reported by Mr. R. Kawamura and by Dr. Ando * (*Rept. Jap. Path. Soc.* v. 6, 1918). On account of these exceptional cases, it will be necessary to forbid the eating of the raw flesh and livers of animals which can harbor the lung fluke.

4. Can the freed larvae infect through mucous membranes outside the digestive tract? To ascertain the pathological changes in the orbits caused by *Paragonimus ringeri*, we dropped normal saline containing larvae just freed from their cysts, on the conjunctiva of dogs,

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* Dr. Ando later repeated this experiment, using eighteen white rats. His results in this second experiment failed to show development in a second host of larvae which had lived for a period in one final host (*Tokyoer Med. Wochenschr.*, No. 2163, 1919).

cats and rabbits. In a little while, we found some small hemorrhages, which were caused by the penetration of the worms. In this experiment, nine rabbits, seven dogs and two monkeys were used, and were examined at various times after these experiments. Each animal showed the presence of the distomes after careful examination first of the orbits and then of the whole body. We proved that the distomes which entered into the orbits were to be found in that place for ten or fifteen days after the beginning of the experiments. But in the cases in which twenty days or more had passed after the experiment, they were found in the chest cavity and not in the orbits. It is very interesting to know how they found their way from the orbits to the chest cavity. We demonstrated this point on experimental animals, and will describe the two most interesting cases.

(a) On September 1, 1918, we put 14 distomes into the right, and 17 into the left orbit of a young dog by cutting open the capsule of Tenon. These distomes were collected from a dog, which was fed with a large number of the encysted larvae sixty-one days before. The dog died on the ninth of that month, eight days after the experiment. On dissection, there was found some muddy exudate in the pleural cavity. The lungs were congested a little and showed some irregular points of hemorrhages here and there, but we could not find any pathological changes, caused by penetration of the worms. We found a distome along the vena cava superior, near the lower end, of the trachea, and another distome on the diaphragm of the right side. In the dissection of the neck we found a distome, which was moving in the loose connective tissue of the right side, about the middle of the trachea, and another distome in the submucosa of the posterior wall of the pharynx. Both eyeballs were badly injured by the operation, but we could not find any worms in the eyeballs and in the orbits. We found only a small suppuration, which was due to the boring of the worms, and the subsequent infection by bacteria, in the tissue of the upper corner of the left orbit and in the left temporal muscle.

(b) On October 11, 1918, we put four distomes into the right and fourteen into the left eye of a big dog by the same operation. These distomes were all mature. The dog died on the twenty-fifth of that month, 14 days after the experiment. On dissection, 2 distomes were found; one of them was situated on the bifurcation of the internal maxillary artery and the superficial temporal artery, and the other worm was located in the masseter muscle around the masseter artery. The lungs and the other viscera were intact.

From these experiments we concluded that the distomes which were dropped on the conjunctiva, penetrate into the orbits and live there a certain number of days. Afterward they escape from the orbits and move to the chest cavity, wandering in the soft tissues.

Therefore, in the monkey's orbits, which are enclosed completely by the bones, they remained a very long time. In the case of a monkey, which was examined eighty-four days after the experiment, we found a living distome in the orbit instead of in the chest cavity.

SUMMARY

1. Young active larvae of *Paragonimus ringeri,* just freed from cysts, cannot penetrate the sound skin, but can enter through a fresh wound.

2. Such young distomes can infect the body by the mouth. Their course from the intestine to the lungs is very similar to the route taken by the encysted larvae.

3. Young distomes just freed from cysts can penetrate the mucous membrane outside of the digestive tract, like the conjunctiva, and bore through the tissues until they reach the lungs.

4. Our experiments indicate that other animals cannot be infected by distomes, which had started development in the final host.

5. Partly developed distomes cannot penetrate the mucous membrane, but, if transferred to the orbits of a suitable host, they penetrate the tissues and reach the lungs.

A NEW NEMATODE FROM THE RAT*

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During the winter of 1920, while I was working at Johns Hopkins University, Dr. W. W. Cort assigned me for investigation the problem of the nematodes of rats and gave me some nematodes, which he had collected from a Norwegian rat, *Epimys norvegicus,* caught in Baltimore. These worms were found to be a new species of Heligmosomum, to which I give the name *Heligmosomum muris* nov. spec. Later I found this same species in twenty-four out of twenty-six rats examined, which were caught near Baltimore. Other nematodes found were *Strongyloides papillosus, Trichosomoides crassicauda, Hepaticola hepatica* and *Heterakis spumosa,* but the present paper describes only *Heligmosomum muris,* since the others have been described by other authors.

Although *Heligmosomum muris* is a common parasite of rats here, nobody seems to have found it previously. My studies on the structure of this species were made for the most part from living material, although specimens preserved in alcohol were used for comparison. The measurements of the body were made on fixed material, because the size of the living worms is changed by movement. Accordingly the sizes given in this paper are a little smaller than those of the living worms.

Specimens of *Heligmosomum muris* preserved in alcohol are dark brown in color, filiform and coiled irregularly two to five times. These worms live in the upper part of the small intestine, especially near the duodenum of the rats. When still in position in the intestine, the worm appears as a little curved red string in the mucus or buried slightly in the mucous membrane. If, however, cool normal saline is poured on the intestine or the rat is not dissected soon after it has been killed, the worms will always be found more or less coiled. In the living worm, the body is red, filiform, and somewhat narrowed anteriorly. The head is small, 21 to 25μ in diameter exclusive of the surrounding cuticular expansion, and 30 to 36μ in diameter including it (Fig. 1). The mouth is small and a small oral cavity (*a*) is present. In an optical section of this region in worms preserved in alcohol the sub-cuticular part of the circumoral area gives somewhat the appearance of two small teeth, one on each side. The esophagus is conoidiform, a little sinous and 0.35 to 0.45 mm. long. The cuticula has transverse striations and prominent longitudinal markings in the form of ten ridges. It is relatively thick and inflated on the head and the anterior cervical region, making a cephalic area or expansion. The length of this area is 0.06 to 0.07 mm. The longitudinal cuticular ridges originate a little behind the cephalic area and run parallel clear to the posterior end. The transverse striations are not continuous around the cuticula but are found only on the longitudinal ridges. The excretory system is well developed and takes the form of two elongate sacs, which contain a large amount of highly refractile granules. These sacs are situated on each side of the body and extend to about the middle of the body. The excretory sacs are connected with the excretory pore by a small canal. The excretory pore is situated on the ventral surface, at a distance of 0.8 to 0.14 mm. in front of the base of the esophagus. The nerve ring is situated just in front of the excretory pore at a distance of 0.2 to 0.25 mm. from the anterior end of the esophagus. Cervical papillae are not present. The cells lining the intestine contain a large amount of melanin-like pigment. This is distributed throughout the entire length of the intestine.

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* This paper is a contribution from the Department of Medical Zoology of the School of Hygiene and Public Health of the Johns Hopkins University.

The male of *Heligmosomum muris* (Fig. 2) is smaller than the female, being 3 to 4 mm. in length with a maximum thickness of 0.085 to 0.1 mm. at the middle of the body. The bursa (Fig. 3) is large and a little enrolled, expanding toward the ventral side. It consists of two large lateral lobes and a small dorsal lobe. The lateral lobes are each supported by six rays and are asymmetrical, the right being larger than the left. In one worm measured, the right lobe was 0.218 mm. long and 0.164 mm. wide, and the left lobe was 0.164 mm. long and 0.146 mm. wide. The rays of the left lobe are more divergent than those of the right, and are different in form on the two lobes. The ventro-ventral, latero-ventral, externo-lateral and medio-lateral rays of the left lateral lobe are similar in form and have almost equal intervals between them. The left postero-lateral ray has a very different form, being shorter and wider than the others and diverges from the medio-lateral ray, curving posteriad. The postero-lateral ray of the right lobe is relatively small and diverges from the medio-lateral ray, curving a little posteriad. The medio-lateral and externo-lateral rays of the right lobe are digital and larger than the other rays. They run close together and parallel throughout most of their extent, but their tips diverge. The latero-ventral ray of the right lobe is long and straight and the right ventro-ventral ray is slender and quite divergent. Both externo-dorsal rays (*ed*) are thin and slender. They diverge from the root of the dorsal ray and run along the boundary line between the two lateral and the dorsal lobes, curving posteriad; consequently they sometimes appear to belong to the lateral lobe and sometimes to the dorsal. The dorsal lobe is very small and divided from the lateral lobes by shallow indentations. The dorsal ray (*d*) is 45 to 54μ long and terminates in four digitations.

The body of the male terminates posteriorly in a thin cone which projects into the bursa along the anterior surface of the dorsal lobe. The two spicules are yellowish brown, about 0.56 mm. long and filiform. They are united at their distal ends and form a small arc. The two gubernacula are colorless and situated on the ventral and the dorsal sides of the distal end of the spicules. The ventral gubernaculum is longer than the dorsal. The former is 0.06 to 0.07 mm. long and the latter 0.04 to 0.05 mm. long.

The anterior part of the testis (Fig. 2, *c*) is situated on the dorsal side near the beginning of the intestine and is loop-shaped. Its beginning is very difficult to see because it is covered with the large excretory sac. However, I could see it sometimes clearly in the living worms, and found that the distance of the anterior tip of the testis from the base of the esophagus varied somewhat in different worms. At the middle of the body, a narrow region (*d*) 0.02 to 0.03 mm. long follows the testis. The wall of this region is thick and consists of cuboidal cells, similar to the cells of the ejaculatory duct. It seems to be a vas deferens because it is joined immediately to the seminal vesicle (*e*) filled with spermatozoa. The seminal vesicle is 0.1 to 0.13 mm. long and is followed by a narrow canal 0.09 to 0.12 mm. long which is surrounded by high columnar cells (*f*). Each of these cells contains a round nucleus and many granules. The cells which are situated on the posterior half of this canal are darker than those of the anterior half. These cells probably correspond to the cement glands of other forms, and the darker coloring appears to be connected with the secretory activity of the cells. This part connects with a well developed ejaculatory duct. The wall of the ejaculatory duct (*g*) consists of a layer of transparent cuboidal cells. At the level of the cement gland the reproductive tube crosses the intestine again. Consequently the anterior half of the intestine is located on the ventral side and its posterior half on the dorsal side of the reproductive tube. The distal end of the intestine joins the posterior end of the ejaculatory duct.

The female (Fig. 4) of *Heligmosomum muris* is larger than the male. It is 4 to 6 mm. long, with a maximum thickness of 0.09 to 0.12 mm. in the middle of the body. In the contracted worm the posterior region of the body is withdrawn to such an extent that the cuticula forms a sac surrounding the anus and vulva. The posterior end of the body becomes reduced suddenly in size just behind the vulva, terminating in a short, thin tail. The anterior end of the ovary (*h*) bends and forms a small loop. This part is situated on the dorsal side of the anterior part of the intestine at a varying distance from the base of the esophagus. The blind free anterior end of the ovary contains extremely small cells, the primordial germ cells. The main part of the reproductive organ is situated on the dorsal side of the body and the ovary is filled with developing oocytes, which generally arrange themselves in single file. The ovary connects with the receptaculum seminalis by a narrow tube near the posterior part of the body. The receptaculum seminalis (*i*) is situated at the beginning of the uterus without sharp demarcation. The uterus is situated in the posterior part of the body and is 0.45 to 0.60 mm. long, containing 13 to 27 eggs, and connects with the ovejector (*j*) of about 0.1 mm. in length. The ovejector is joined to the thick walled vagina, crossing the distal end of the intestine. The ovejector has a well developed wall which at the beginning of the ovejector is thickened forming a sphincter. The ovejector seems to be a little twisted and its distal end projects into the vagina. The vagina (*k*) is 0.14 to 0.16 mm. long and situated on the ventral side of the body. Its wall is lined by cuticula. There are strong irregular longitudinal folds on its internal surface and it extends into the distal end of the ovejector. The vagina runs in a diagonal direction in the posterior end of the body, and terminates in the vulva. This (*l*) is situated on the ventral surface just in front of the anus at a distance of 0.1 to 0.13 mm. from the tip of the tail. The intestine is straight and runs along the ventral side of the body, terminating in the anus. It crosses the ovejector near the posterior end of the body. The anus (*m*) is situated at a distance of about 0.06 mm. from the tip of the tail. The eggs are ellipsoidal with a very thin shell. The average size is 58μ by 33μ; a common minimum is 54.6 by 30.9μ and a frequent maximum 61.8 by 34.5μ. They are in the one to sixteen cell stages of development in the uterus and in the feces are in the four to sixteen cell stage, exceptionally, also in the morula stage.

In the genus Heligmosomum, Raillet and Henry the following species have been placed: *Heligmosomum costellatum* (Dujardin), *H. minutum* (Dujardin), *H. gracile* (F. S. Leuckart), *H. laeve* (Dujardin), *H. braziliense* Travassos, *H. agoutii* Neiva, da Cunha and Travassos, *H. vexillatum* Hall and *H. cristatum* Gedoelst. All of these are discussed by Hall (1916: 149-158) except *H. agoutii* Neiva, da Cunha and Travassos (1914) and *H. cristatum* Gedoelst (1917). Seurat (1915) gives a detailed description of *H. laeve* Dujardin, which is not fully covered in Hall's paper. *Heligmosomum muris* is most closely related to *Heligmosomum vexillatum* Hall, which was described from *Thomomys fossor* from Colorado, and to *Heligmosomum brasiliense* Travassos from the Norwegian rat from Rio de Janeiro, Brazil. A comparison of *H. muris* with the description of *H. vexillatum* and with a toto mount of this species which was kindly loaned me by Doctor Hall, showed that while these two species agree in general size and shape, they are strikingly

YOKOGAWA—NEW NEMATODE FROM THE RAT

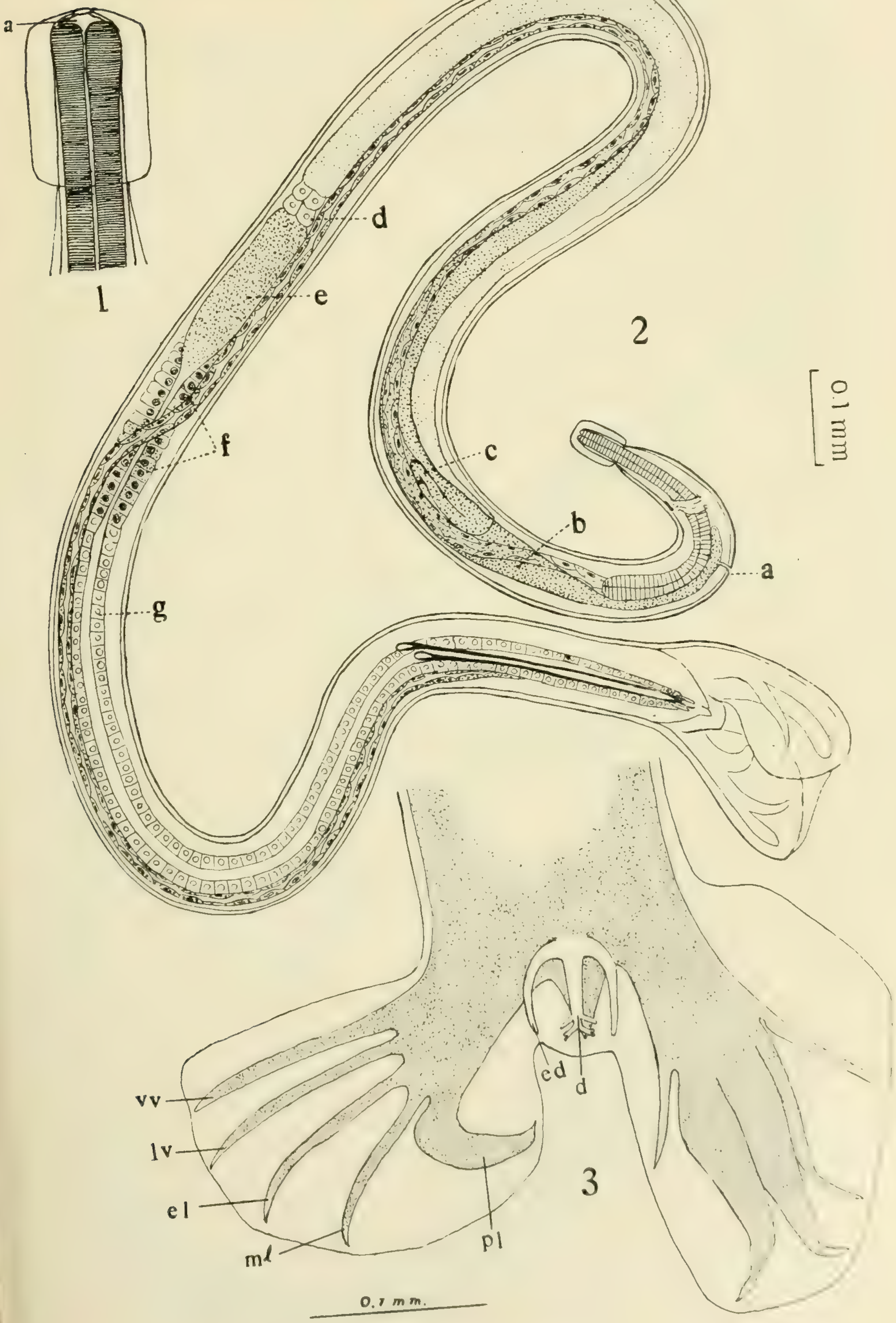

PLATE VI

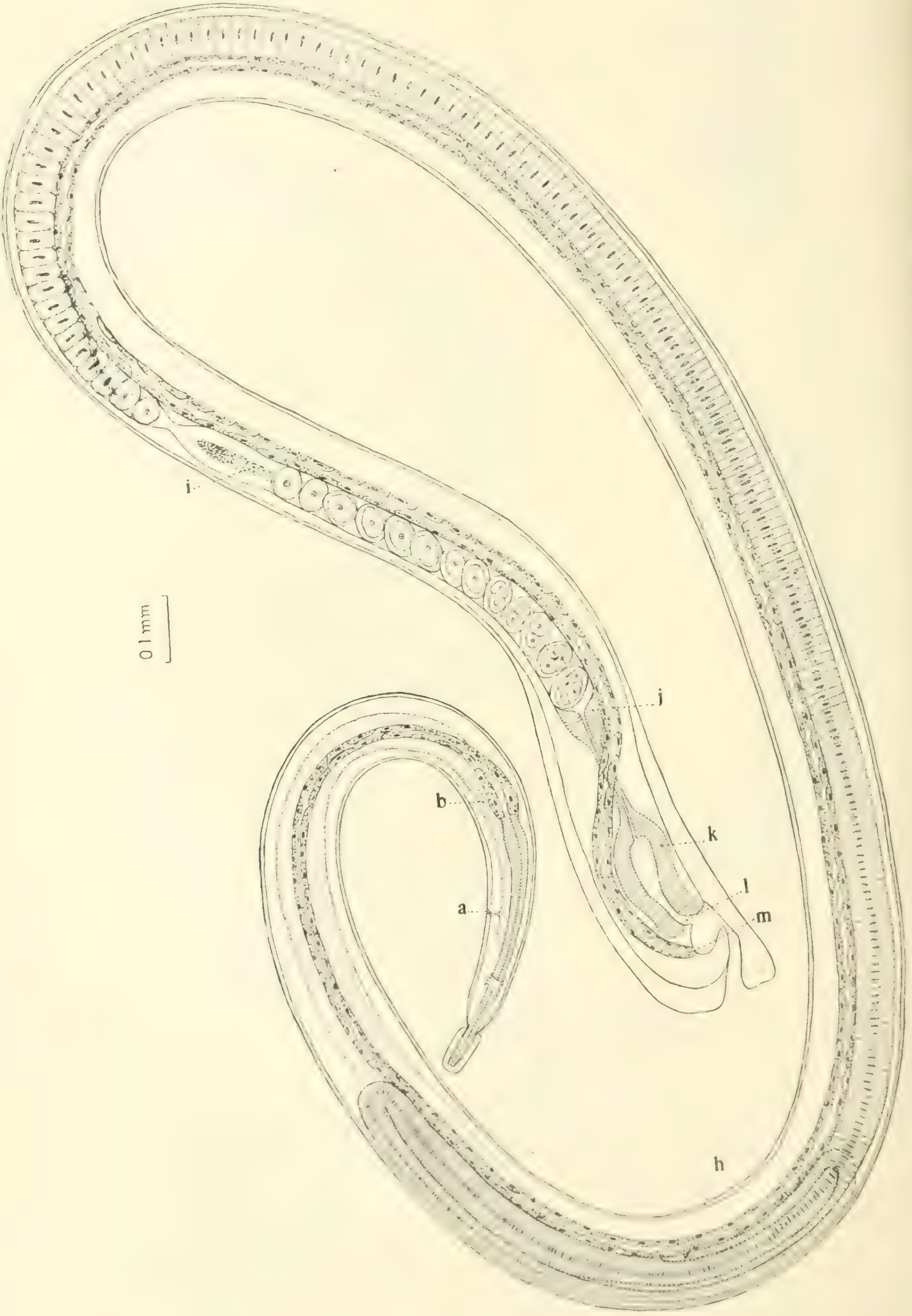

PLATE VII

different in the length of the spicules, the size of the eggs, and the character of the bursae. A comparison of my species with *H. braziliense* is more difficult since this species is not figured and not described in detail, especially in regard to the character of the bursa. Differences are apparent in the size of the eggs, the length of the esophagus, the structure of the bursa, and the position of the anus and vulva. The most striking differences are in the size and shape of the two species. In *H. braziliense* the male is from 2.6 to 2.8 mm. in length and 0.09 to 0.1 mm. in diameter at its widest part, giving the ratio of length to width of about 30: 1. The female is 3.5 mm. long and 0.13 mm. in width with a ratio of length to width of about 27: 1. *H. muris* is longer and especially in the female much narrower for its length, as in this species the male has a length of 3 to 4 mm. and a width of 0.85 to 0.1 mm., making a ratio of length to width of about 33 to 44: 1, and the female has a length of 4 to 6 mm. and width of 0.09 to 0.12 mm., making a ratio of length to width of 44 to 50: 1. These differences seem to me to make it necessary to establish *Heligmosomum muris* as a distinct species.

I wish to express here my deep indebtedness to Doctor W. W. Cort, under whose direction this work was carried on, and also to Doctor B. H. Ransom and Doctor M. C. Hall for their kindness in helping me with the preparaton of this paper.

PAPERS CITED

Gedoelst, L. 1917.—Nematodes parasites du *Sciurus prevosti* de Sumatra. Rev. zool. Africaine, 5: 153-162.

Hall, M. C. 1916.—Nematode parasites of mammals of the orders Rodentia, Lagomorpha, and Hyracoidea. Proc. U. S. Nat. Mus., 50: 1-258.

Neiva A., da Cunha, A. M., and Travassos, L. 1914.—Parasitologische Beitrage. Memorias do Instituto Oswaldo Cruz, 6: 180-191.

Seurat, L. G. 1915.—Sur les conditions de la ponte du strongle lisse. Bull. scient. France et Belg., 48: 171-177.

Travassos, L. 1914.—Tricostrongylidoes brazileiros. Brazil medico, 28: 325-327.

EXPLANATION OF PLATE VI

Fig. 1.—The anterior end of *Heligmosomum muris*, shown in optical section; *a*, mouth cavity.

Fig. 2.—Male of *Heligmosomum muris* from an experimental rat, 9 days after infection, latero-ventral view; *a*, excretory pore; *b*, excretory sac; *c*, anterior end of the testis; *d*, vas deferens; *e*, seminal vesicle; *f*, cement gland; *g*, ejaculatory duct.

Fig. 3.—Bursa of male of *Heligmosomum muris*, dorsal view; *d*, dorsal ray; *ed*, externo-dorsal ray; *pl*, postero-lateral ray; *ml*, medio-lateral ray; *el*, externo-lateral ray; *lv*, latero-ventral ray; *vv*, ventro-ventral ray

EXPLANATION OF PLATE VII

Fig. 4.—Female of *Heligmosomum muris* from the wild rat. *a*, excretory pore; *b*, excretory sac; *h*, anterior end of ovary; *i*, seminal receptacle; *j*, ovejector; *k*, vagina; *l*, vulva; *m*, anus.

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On the Migratory Course of *Trichosomoides Crassicauda* (Bellingham) in the Body of the Final Host

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ON THE MIGRATORY COURSE OF *TRICHOSOMOIDES CRASSICAUDA* (BELLINGHAM) IN THE BODY OF THE FINAL HOST *

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Trichosomoides crassicauda, a nematode belonging to the family Trichinellidae, was found in the bladder of wild rats in 1845 by Bellingham. Hall (1916) has summed up the previous work on this species. This nematode is remarkable on account of the great difference in size between the males and females. The male is commonly found parasitic in the vagina or uterus of the female, having a length of 1.46 to 2.5 mm. and a width of 23 to 33μ. The female is 10.5 to 13 mm. long and attains a maximum width in the posterior region of the body of about 0.2 mm. Nothing is known of the method of infection of this parasite or its migratory course to the bladder of the rat. Von Linstow (1874) suggested that the embryos might bore into the wall of the digestive tract and make their way to the pelvis of the kidney by way of the renal artery. He described also that sexually immature individuals, the males but little smaller than the females, were found in the pelvis of the kidney and that copulation took place in the ureters. Later the females became larger and the males entered their vaginae. Hall (1916: 16) found that the embryos escaped from their shells in the vagina of the female after the worm had been in normal salt solution a short time. He suggested from this observation and from the fact that such embryos seemed to live only a short time that infection must take place in a rather short period as a rule, or else the embryos would perish. He also described that the embryos which had just escaped from the egg, had a body of almost uniform thickness, terminating in bluntly rounded ends, while von Linstow stated that they were provided anteriorly with a single lancet-like process.

I found some specimens of *Trichosomoides crassicauda* in wild rats *Epimys norvegicus,* collected near Baltimore during the winter of 1919-20. The parasites were usually found attached to the wall of the bladder with their anterior ends somewhat embedded in the mucous membrane. They cause a rather high degree of catarrhal cystitis according to the number of the parasites present. The mucous membrane of the bladder was found congested and swollen and the urine more or less muddy. The number of the parasites in the bladder of a single rat is commonly small, although sometimes ten or more specimens were found in a single host.

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* Contribution from the Department of Medical Zoology of the School of Hygiene and Public Health of the Johns Hopkins University

In order to discover the method by which the rat is infested with *Trichosomoides crassicauda* I fed two white rats on January 20, 1920, with the eggs which contained the fully developed embryos. One of these rats became infected and the parasites developed to maturity. The eggs from the urine of this rat were used in later experiments. After the success of this experiment I undertook to investigate the migratory course of the larvae in reaching the bladder of the rat by feeding white rats with large numbers of the eggs of this parasite, and examining the various organs a short time after feeding. Three rats were used at different times but from only one of them were the larvae recovered. The results of this study are incomplete and a much larger series of experiments will be needed to clear up the details of the problem. However, some very definite information was obtained in regard to the migratory course of this parasite in the body of the final host. Since it will be impossible for me to continue these experiments I am writing up my results in hopes that some one else may use them in carrying on further work on this interesting problem.

Since the methods used with the three experimental rats were the same, I will describe here only the details of the work with the one rat which gave positive results.

On May 15 and 16, I fed one half grown white rat with many eggs of *Trichosomoides crassicauda* collected from the urine of the white rat experimentally infected with the parasite and of two wild rats which were infested naturally. The next day I fed it 15 adult worms containing many eggs in their uteri and also many eggs collected from the urine of the three rats mentioned above. On the following day also it was fed with many eggs collected from the urine of these rats. I killed this rat on May 19, one to four days after the various feedings.

The following technic was used in the recovery of the larvae from the body of the rat. First I opened carefully the abdominal cavity, avoiding bleeding, and washed it out several times with normal saline for the purpose of collecting any larvae contained in it. The liver and other internal organs were found to be a little congested without showing any bleeding points. Then I opened the pleural cavity in the same way and washed it out with normal saline. Both lungs were found to be a little congested showing some bleeding points on their surfaces. I examined one third of the liver, both kidneys, the spleen, and the left lung according to the following method. These internal organs were crushed into fine pieces and washed with normal saline two to four times, and filtered through a fine wire net. The filtrate from the different organs and wash water of abdominal and pleural cavities were kept carefully separate and were centrifuged to collect the larvae.

The following results were obtained from these examinations. Four larvae were found in the abdominal cavity, two in the pleural cavity, and three in the left lung. One third of the liver, both kidneys and the spleen were examined without finding any larvae, while the right lung and two thirds of the liver were preserved for later microscopical examinations. I also examined the ureters, bladder and heart, without finding any larvae. Comparing the structure of the young larvae found in the body cavity and in the lungs with larvae just escaped from the eggs, I learned the following facts. The larvae just from the eggs have a very small body of almost uniform thickness, terminating in bluntly rounded ends. They measure about 0.21 to 0.25 mm. in length and 8 to 10μ in thickness. It was impossible to make out any details of internal structure at this stage. The larvae found in the abdominal cavity of the experimental rat measured 0.82 to 0.84 mm. in length and 34 to 35μ in width. The anterior part of the body was a little thicker than the posterior end and ended bluntly. The digestive tract was not very clearly defined. The posterior end of the body was blunt with a diameter of about 0.02 mm. and had a small depression. The esophagus consisted of about twenty irregularly shaped cells. No esophageal cells were present for about 0.02 to 0.025 mm. at the anterior end. The esophagus was relatively long, measuring about 0.28 mm. The intestine ran straight along the middle body toward the posterior end and consisted of cells containing fine granules. The larvae found in the pleural cavity and two of the larvae found in the lungs were larger than those from the abdominal cavity. They were about 2.34 mm. long and 0.1 mm. thick. The anterior end was rounded and 0.03 mm. in diameter. The width increased toward the middle of the body with a maximum of 0.1 to 0.11 mm. and then decreased posteriad. The esophagus consisted of about twenty cells and measured about 0.54 mm. in length. No cells were present for a short distance at the anterior end. One larva found in the lung was smaller than the others found in the same location, having a similar size and structure to the larvae found in the abdominal cavity. It is very probable that the larger larvae are females and the smaller ones males.

From these experiments I learned the following facts (1) Infection with *Trichosomoides crassicauda* can be induced in the white rat by feeding the eggs of the parasite (2) The adult worms found in the bladder of experimental animals are very few compared with the number of eggs swallowed. (3) The eggs swallowed by the final host hatch in the digestive tract and penetrate through its wall into the abdominal cavity. From here they travel into the pleural cavity probably through the diaphragm and penetrate into the lungs from their surfaces.

The passage of the larvae of *Trichosomoides crassicauda* into the lungs of the experimental rat is very interesting in relation to the recent work on the life history of *Ascaris lumbricoides* which proves that after hatching in the intestine of the final host the larval stages of this parasite must make their way to the lungs before they can complete their development. Yoshidi (1918) and Ransom and Foster (1920: 30) conclude that this phenomenon is of common occurrence in the life cycles of parasitic nematodes. Besides the hookworms, Strongyloides, *Ascaris lumbricoides, Ascaris suum* and *Belascaris marginata,* they note also that *Haemonchus contortus* from the sheep and *Ascaris anoura* from the python probably have a lung phase in their life cycles. Recently Neshi (1918) reports the finding of four larvae of *Trichuris depressiuscula* in the lungs of a dog twenty-one hours after experimental infection.

This observation and my finding the larvae of *Trichosomoides crassicauda* in the lungs of the experimental rat after feeding with the eggs add the family Trichinellidae to those in which this phenomenon occurs, and strengthens Ransom and Foster's hypothesis that parasitism of the lungs by nematodes is a more primitive condition than parasitism of the alimentary canal.

On the method of migration of the larvae of this nematode from the lungs to the bladder of the host there is little definite information. Von Linstow's (1874) finding young worms in the kidneys and ureters suggests that they make their way to the kidneys and then pass down the ureters to the bladder. How they make their way from the lungs to the kidney is still an unsolved question. Taking for granted that the migration to the lungs is a necessary phase of the life history of this parasite, there are three possible ways in which it might migrate from the lungs to the kidneys. (1) The larvae might make their way into the small branches of the pulmonary veins, be carried to the heart and then pass to the kidney along the aorta and renal arteries. (2) The larvae in the lungs might break into the air cells and, like the hookworm larvae, travel up the trachea and down the esophagus into the intestine. From here it would be necessary for them to make their way to the kidneys by way of the body cavity. (3) Finally the larvae in the lungs might make their way back into the pleural cavity, through the diaphragm and body cavity to the kidney. The course by the blood stream would seem to be very difficult if not impossible on account of the large size of the larvae and the fact that the renal artery is a small vessel which branches at right angles from the dorsal aorta. It is possible of course that the smaller type found might follow this course. It seems to me that the second course is the most probable, but the solution of this interesting problem must await future investigations. At any rate the passage to the kidney requires a prolonged and difficult migration. This may account for the difficulty of producing the infestation and why the number of worms found in the bladders of the wild rats is usually so few.

SUMMARY

1. The infestation of rats with the bladder nematode, *Trichosomoides crassicauda,* was accomplished by feeding the eggs.

2. The finding of larvae of this species in the body cavity, pleural cavity and lungs of an experimental rat fed with large numbers of eggs, suggests that in the life cycle of this species the larvae must pass to the lungs before they can establish themselves in their normal habitat.

3. This observation and other recent studies strengthen the view that migration to the lungs is a common phenomenon in the life cycle of nematodes.

4. How larvae reach the bladder from the lungs was not determined but they probably are not carried in the blood vessels.

I wish to express here my thanks to Dr. W. W. Cort, under whose direction this work was carried on, for his help in the course of this investigation and for revising the manuscript.

LITERATURE CITED

Hall, M. C. 1916.—Nematode Parasites of Mammals of the Orders Rodentia, Lagomorpha, and Hydracoidea. Proc. U. S. Nat. Mus., 50: 1-258.

Neshi, G. 1918.—Ueber die Entwicklung des Trichocephalus innerhalb des Wirtes. (Japanese.) Tokyoer Med. Wochr., No. 2080.

Ransom, B. H., and Foster, W. D. 1920.—Observations on the Life History of *Ascaris lumbricoides.* U. S. Dept. Agr. Bull. 817.

Von Linstow, O. 1874.—Beobachtungen an *Trichodes crassicauda* Bell. Arch. Naturg., 1: 271-286; 1 pl. (Cited after Hall, 1916.)

Yoshida, S. 1918.—The Migratory Course of the Larvae of Ascaris. (Japanese.) Byo ri Gaku Zasshi, Vol. 8.

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AN EXPERIMENTAL STUDY OF THE INTRACRANIAL PARASITISM OF THE HUMAN LUNG FLUKE, *PARAGONIMUS WESTERMANII*.*

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(Received for publication September 18th, 1920.)

Introduction.

Cerebral parasitism by the human lung fluke, *Paragonimus westermanii* was first recognized by Otani (1888–1889) in 1887. This author described the case of a man who, after suffering for a long time with paragonimiasis of the lungs, became suddenly afflicted with general epilepsy. The patient died after a lapse of one and one-half years, and the autopsy disclosed one tumor, as large as a hen's egg, and several smaller ones in the occipital and the frontal lobes of the brain. In the larger tumor were found two adult lung flukes and some yellowish brown material, consisting of many eggs of the worms, Charcot-Leyden crystals and hematoidin crystals. Afterwards (1890–1891) he reported the clinical histories of three similar cases.

Following this work 19 additional cases have been reported by Otani (1888), Katsurada (1900), Taniguchi (1904), Misumi (1907), Musgrave (1907), Tsunoda (1908), Karasawa (1910), Kimura (1913), Inamoto (1917) and Kawamura (1918). In all of these the presence in the brain, of the parasite or its eggs was established at autopsy. The authors mentioned have discussed particularly the relation of this type of parasitism to Jacksonian epilepsy and other prominent nervous symptoms. An analysis of these cases will be given later in this paper.

The hypothesis that the worms and their eggs were carried from the lungs to the brain by means of the blood, was formerly accepted by all authors. The pathological changes in the brain were believed to be due to the production of anemic infarcts referable to emboli of eggs or worms (Katsurada (1900), Taniguchi (1904), Tsunoda (1908), Yamagiwa (1892). The senior author (Yokogawa, 1916) in an extensive series of experiments has proved, however, that the lung fluke in its migrations in the final host always travels through the soft tissues and is never carried by the blood stream. In the course of these experiments he found four partially developed worms traveling through the loose connective tissue along the blood vessels and nerve trunks in the necks of two dogs. The position of these worms suggested that the course of the parasites from the chest cavity to the cranial cavity is along the soft connective tissue of the neck and through the jugular foramen. Later Kawamura and Matsui * (1916) and Ando (1917) also reported the finding of worms, in their own experiments, in the same location as those which Yokogawa found. No one, however, had been successful in producing intracranial parasitism by this worm experimentally. The experiments described in this paper were undertaken for the purpose of studying the pathological changes produced in the brains of experimental animals as a result of cerebral paragonimiasis, and also with the hope of discovering the course which the lung fluke takes in migrating to the brain.

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* A contribution from the Department of Pathology of the Medical College of Formosa and the Department of Medical Zoology of the School of Hygiene and Public Health of The Johns Hopkins University.

All the dogs, used in these experiments came from the city of Taihoku, Formosa, where the work was carried on, or from the districts close to this city. Previous examinations had shown that *Paragonimus westermanii* is rare among the dogs of this region. As an additional precaution, however, the dogs were kept for a sufficient length of time to allow any parasites that might have been present to develop to maturity. A series of fecal examinations for the eggs of the parasite was made in the case of each dog before it was used for the experiment. Further, since the degree of development of the parasites for various periods of time had been established by previous experiments it was possible to check the source of any parasites found in the course of the experiments by the degree of growth at the time of observation. It would accordingly seem extremely improbable that the results obtained could have been complicated by previous infection of any of the dogs.

Three series of experiments were carried out: The first series was performed with the idea of ascertaining whether the course of the fluke toward the brain was by way of the connective tissues, and whether entrance into the cranial cavity was effected through the foramina. The second series of experiments was made to test the hypothesis held by certain authors that in cases of cerebral parasitism by the lung fluke the parasite is carried to the brain by the blood stream. In the third series of experiments both immature and adult flukes were introduced directly into the cranial cavity by trephining the skull, for the purpose of determining what pathological changes they would produce in the brain and what course they would follow in their migrations. An analysis of the case records of human cerebral paragonimiasis has finally been made for the purpose of ascertaining whether the data regarding the pathological changes produced in the brain and the position of the parasites furnish any clue to the course which they follow in migrating to the cranial cavity.

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* This was reported by Dr. Kawamura at the meeting of the Japanese Pathological Society in Tokyo, in April of 1916.

I. EXPERIMENTS TO TEST WHETHER IMMATURE LUNG FLUKES WILL ENTER THE CRANIAL CAVIITY THROUGH THE ANATOMICAL APERTURES.

Experiment 1.—Immature distomes which had been undergoing development for varying periods of time in other animals were introduced into wounds in the sides of the necks of five dogs. The number of flukes used for each experimental dog and the age which they had reached in other animals was as follows: for dog number one, eleven flukes, twenty days old; for dog number two, eleven flukes, twenty days old; for dog number three, eighteen flukes, eighteen days old; for dog number four, nine flukes, twenty-three days old; and for dog number five, fifteen flukes, forty-two days old. The dogs were examined at intervals varying from one to fifteen days following the operation, but none of the young distomes had entered the cranial cavity. In three of the dogs, however, worms were found in the pleural cavity, at the time of examination. The negative results of this experiment while suggestive do not prove that the lung flukes may not enter the cranium through the anatomical apertures, since the experimental conditions were, of course, very different from a natural process, and since the interval between the introduction of the flukes into the dogs and the examination was a short one owing to the development of severe suppuration in the area of operation.

Experiment 2.—Young distomes which had just emerged from their cysts were introduced into the orbits of eight dogs, through the conjunctiva, to determine whether they would enter the cranial cavity by passing through the superior orbital fissure or the foramen nervi optici. Table I gives the data obtained in this experiment. Although this experiment was not far removed from a natural process, in no case were the worms found to have penetrated into the cranial cavity. The failure of the young distomes to take this course is probably due to the fact that the orbital walls in dogs are incomplete. This gives the worms a chance to escape through the soft tissues of the external wall of the orbit, without penetrating the cranial cavity. That this is actually what happened is indicated by the finding of worms in the pleural cavities and lungs of five of the eight dogs (see Table I).

TABLE I.

Data obtained as the result of the introduction of worms which had just escaped from their cysts, into the orbits of dogs.

Serial number of dogs used.	Number of young worms introduced into orbits.		Number of days from introduction of worms until examination of hosts.	Locations in which worms were found at examination.
	Right orbit.	Left orbit.		
I	20	8	5 days	In left orbit
II	10	10	11 days	In left orbit
III	—	20	15 days	One in pleural cavity
IV	13	17	22 days	None found
V	28	21	68 days	Two in pleural cavity
VI	50	64	74 days	Two in pleural cavity
VII	22	23	127 days	Six in lungs
VIII	44	39	201 days in right orbit, 177 days in left orbit	Three in pleural cavity

II. EXPERIMENTS UNDERTAKEN TO DETERMINE WHETHER YOUNG LUNG FLUKES, JUST FREE FROM THEIR CYSTS, CAN BE INTRODUCED INTO THE CRANIAL CAVITY BY WAY OF THE INTERNAL CAROTID ARTERY.

Young distomes, which had just escaped from their cysts were placed in normal saline and injected into the common carotid artery, of eight dogs. The results obtained are shown in Table II. In order to insure that these worms would enter the internal carotid artery and thus be carried to the brain a ligature was placed upon the common carotid artery just beyond the lingual branch in dogs IV and V, while in dogs VI, VII and VIII all the branches of the common carotid artery except the internal carotid artery were tied.

As shown in Table II no worms were found in any case in the cranial cavity, while in four of the eight dogs they were met with elsewhere in the body. The findings in the last 3 cases, as recorded in this table, are especially significant, as they leave no doubt that the large number of worms injected was actually carried by the internal carotid artery to the brain. There were no gross pathological changes produced in the brains of any of the dogs used in this series of experiments. That the worms had actually entered the brain substance was shown by a thorough microscopical examination of the brain of the last dog, which disclosed hemorrhagic infiltrations and many irregular areas of destruction of the cerebral substance. These

TABLE II.

Data from the experiments on the introduction of young flukes, just freed from their cysts, into the cranial cavity through the internal carotid artery.

Serial number of dogs used.	Interval between the introduction of the worms and the examination of the dogs.	Number of worms injected.	Place of injection.	Arteries ligated.	Location of worms found.
I....	8 days	13	Left common carotid artery	—	—
II....	270 days	32	Left common carotid artery	—	8 worms in the pleural cavity
III..	24 days	18	Left common carotid artery	—	—
IV....	27 days	43	Right common carotid artery	Common carotid artery beyond lingual artery	6 in the pleural cavity, 1 in the abdominal cavity
V...	77 days	45	Left common carotid artery	Common carotid artery beyond lingual artery	—
VI....	13 days	117	Left common carotid artery	All branches of the common carotid artery, except the internal carotid artery	—
VII....	12 days	112	Right common carotid artery	All branches of the common carotid artery, except the internal carotid artery	1 in the pleural cavity
VIII...	46 days	89	Left common carotid artery	All branches of the common carotid artery, except the internal carotid artery	3 in the pleural cavity

changes could only have been produced by the young distomes in the brain. These findings therefore raised three questions: (1) Why were there no gross pathological changes in the brains; (2) why did the worms not remain and develop in the cranial cavity, and finally (3) by what route did they travel from the cranial cavity to the chest cavity? An attempt will be made in the remainder of the paper to answer these questions.

III. EXPERIMENTS MADE TO DETERMINE WHAT CHANGES TAKE PLACE IN THE BRAIN, WHEN SPECIMENS OF *Paragonimus westermanii*, IN VARIOUS STAGES OF DEVELOPMENT, ARE INTRODUCED DIRECTLY INTO THE CRANIAL CAVITY OF DOGS BY TREPHINING THE SKULL, AND WHAT COURSE THEY TAKE IN MIGRATING FROM THE CRANIAL CAVITY TO THE CHEST CAVITY.

Since it has been found impossible to produce intra-cranial infestation experimentally either by the introduction of the young worms into wounds in the necks of dogs, or by way of the orbits, or by the internal cartoid artery, a series of experiments was undertaken, in which both young and fully developed worms were introduced directly into the cranial cavity by trephining the skull. The technic followed was that usually employed in such operations.

After exposure of the dura mater, this membrane was incised and the worms were placed upon the pia mater. The cut edges of the dura were held apart and the worms observed until they had disappeared through the pia mater. The opening in the skull was then closed with paraffin and the skin sutured. In introducing the adult worms the operation was the same, except that the dura mater had to be raised a little to make a space for the worm between it and the pia mater. In all, six dogs were used in this experiment. On account of the special significance of the findings obtained in these experiments a detailed account will be given of each.

Experiment 1.—On December 17, 1918, 45 lung fluke larvae, just freed from their cysts, were introduced under the dura mater of a dog by following the operative procedure described above. On January 9, 1919, i.e., after about 23 days, the dog was found dead. An examination showed that the wound made at the operation was completely healed, and that the opening in the skull was also entirely closed by the paraffin. A part of the dura mater, at the point of operation appeared roughened, a little thickened and congested. No striking changes were noted in the brain substance, although there was a little congestion. We could not find any worms in the cranial cavity. Two distomes, however, were encountered in the pleural cavity. On the surface of both lungs there were several ecchymotic points, and in the lungs several small greyish resistant areas without air.

Experiment 2.—A dog was operated on December 17, 1918, and 36 larvae, just free from their cysts, were introduced under the dura and the wound closed, as described above, after the worms had been seen to penetrate into the brain, through the pia mater. On January 15, 1919, i.e., 27 days after the operation, the dog was killed, and carefully dissected. A part of the dura mater which corresponded to the trephined area was found firmly adhering to the skull, and contained a number of grey or yellow granules. There were no striking changes in the brain except several small traumatic areas on its surface, which corresponded to the place of the operation. In the water, in which we washed the brain, was found a single distome, and eighteen more distomes were encountered in the pleural cavity. The worms found in the pleural cavity were in the normal stage of development corresponding to the time represented in this experiment, but the single specimen in the brain, although introduced at the same time as the others, was very much smaller. Both lungs were somewhat congested and showed many irregular areas of ecchymosis on their surface.

Experiment 3.—A dog was operated on December 20, 1918, and 16 distomes, which had just emerged from their cysts, were introduced under the dura mater, as in the preceding experiments. On January 15, 1919, i.e., 26 days after the operation, this dog was killed. We could not find any worms either in the cranium or in the chest, nor could we notice any changes in the brain caused by the distomes.

Experiment 4.—On February 27, 1919, a dog was operated on and 18 larvae which had just emerged from their cysts were placed under the dura mater, as in the previous experiments. This dog was killed on March 4, only 5 days after the operation. The wound was not yet completely healed. The membranes and the brain itself showed no striking changes, although there was a somewhat greater degree of congestion than normal. No distomes were found in the cranial cavity, but one was met with in the pleural cavity.

Experiment 5.—A dog was operated on March 4, 1919, three full-grown distomes which had developed in a cat, were introduced under the dura mater, and the wound closed. The dog was killed on the seventeenth of the same month, i.e., 13 days after the operation. Although the wound had suppurated and a purulent inflammation, the result of the operation, had developed in the right temporal muscle, yet the opening made by trephining was found to be closed by a membrane of connective tissues. On opening the cranium, we found that the skull contained much blood. The dura was generally congested and adhered firmly to the skull in the right parietal region, and to the pia mater of the same region. This adhesion corresponded to the area which had been trephined, and appeared a little thickened and opaque. There was an ecchymotic area, the size of a one cent piece, in the cortical substance of the right frontal lobe, and another as large as a pea, in the cerebral substance in the middle region of the right parietal lobe. Other parts of the cerebrum, as well as the cerebellum and the medulla oblongata, showed no striking changes. Both lungs were free from adhesions, but in the posterior upper border of the lower lobe of the left lung, we felt a resistant area which seemed embedded in normal tissue. The surface of the lung in this region was smooth and normal. When the resistant area was dissected out we found a circumscribed hemorrhagic cyst, containing a full-grown distome. There was no development of connective tissues in the wall of this cyst.

Experiment 6.—A dog was operated on March 4, 1919, and three full-grown distomes which had developed in a cat, were introduced under the dura mater. This dog was killed on the seventeenth of the same month, i.e., thirteen days after the operation. The wound had healed completely. There was a small area of ecchymosis in the right temporal muscle. The bone and the dura mater were hyperemic. The sagittal sinus, especially on its right side, was highly congested. The right half of the dura mater adhered slightly to the skull by fibrous tissue and in the parietal region of the same side was found a dark brown spot as large as the tip of a man's finger. The pia mater was also congested, especially over the anterior half of the right parietal lobe. The external surface of the temporal lobe adhered widely to the dura mater, and contained a greyish lump. Although this lump gave the appearance of a dead distome, we could not be certain of this from examination. The substance of the brain was a little softened, but there were no other striking changes. On the inferior surface of the upper lobe of the left lung there was an ecchymotic area, as large as a pea, and on the upper part of the posterior surface of the left lower lobe, one as large as the tip of a man's thumb. In the latter region we felt a resistant area which contained a full-grown distome. Another full-grown worm was found in the right pleural cavity and in the right lung there were several ecchymotic spots of various sizes, which were apparently caused by this worm.

In Table III is presented a summary of the six experiments described above, including a statement of the place on the skull where the operation was performed.

TABLE III.

No. of experiment.	Duration of infestation.	Number of larvae introduced.	Adult worms introduced.	Trephined area.	Gross pathological changes in the brain.	Location of worms found.
I......	23 days	45	—	Left parietal region	–	2 in the pleural cavity
II......	29 days	36	—	Left parietal region	–	18 in the pleural cavity; 1 in the brain
III......	26 days	16	—	Left frontal region	–	—
IV......	5 days	18	—	Left frontal region	–	1 in the pleural cavity
V......	13 days	—	3	Right parietal region	+	1 in the lung
VI......	13 days	—	3	Right parietal region	+	1 in the lung; 1 in the pleural cavity

An analysis of the experiments just described makes it possible to draw certain definite conclusions regarding intra-cranial infestation on the part of *Paragonimus westermanii.* One of the most surprising findings is the fact that the pathological changes which result from the introduction of the worms into the cranial cavity of dogs, are remarkably slight, especially when compared with the changes that have been described by various authors in cases of cerebral paragonimiasis occurring in man. Two explanations might be offered to account for this difference. In the first place, the time spent by the worms in the brains of the dogs was very short, while in the human cases there is every indication that the condition was of much longer standing. In the second place the tissues of the dog, especially the brain, do not react as strongly to injury as do those of man. This is shown by the fact that the trephining operation is so much less severe for the dog than for man. On the day following such an operation the animal will stand up and begin to take food.

Another point which is clearly brought out by these experiments is the fact that the cranial cavity is not a favorable location for the development of *Paragonimus westermanii.* This view is of course strongly supported by the relative infrequency of cerebral paragonimiasis in man. The finding, in the second experiment of the series, of a young distome in the cranial cavity, twenty-nine days after the operation throws light on this question. This worm showed considerable retardation in its development, being much smaller than those of the same age which were found in the pleural cavity of the same experimental dog. The rapidity with which the worms introduced into the cranial cavity migrated from this location also suggests that it is an unfavorable environment for their development.

As in the above experiments the worms which were introduced into the cranial cavity were later found in the pleural cavity or the lungs, the question of the course which they followed in this migration is of course immediately raised. There are two possible routes which suggest themselves: (1) The worms may have left the cranial cavity by way of such anatomical apertures as the posterior fissure or the inferior orbital fissure and may have reached the chest cavity by traveling through the soft tissues, especially the connective tissue along the large blood vessels or nerves; or, (2) they may have gotten into the cerebral sinuses and have been carried to the lungs by way of the internal jugular veins, the right ventricle and the pulmonary arteries. It was found that the young distomes which were introduced into the orbits of dogs and which had migrated to the pleural cavity and the lungs, without entering the cranium, needed from 8 to 15 days to complete their journey. This being the case, a longer period of time even would seem to be necessary for worms, placed on the surface of the brain, to reach the lungs, if they escaped from the anatomical apertures and migrated through the tissues of the head and neck. In this connection experiment No. 4 is of special interest. In this case only five days elapsed from the introduction of the worms into the cranial cavity until one was recovered from the pleural cavity. In the light of previous knowledge it would seem to be impossible that migration through the tissues could be accomplished in such a short time.

Of further significance in this connection are the findings recorded in the case of the fifth experimental dog. In this animal a full grown distome was found in a fresh hemorrhagic cyst, situated near the upper posterior edge of the lower lobe of the left lung. The condi-

TABLE IV.

Analysis of the position in the brain of the pathological changes found in the reported cases of human cerebral paragonimiasis.

Reporter			Otani, S.	Katsurada, F.	Taniguchi, N.	Misumi, J.			Tsunoda, G.	Karasawa, M.	Inamoto, K.								Kimura, D.	Kawamura, R.	Total 18.	
Serial number of cases			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII		
Location of foci of infestation with *Paragonimus westermanii* in the human brain.	Frontal lobe	Left				+					+									+	3	9
		Right	+		+				+		+					+				+	6	
	Parietal lobe	Left					+				+			+							3	5
		Right			+				+												2	
	Temporal lobe	Left		+		+	+		+			+				+		+		+	8	12
		Right			+				+	+	+										4	
	Insula	Left																				2
		Right						+		+											2	
	Occipital lobe	Left				+	+					+	+		+		+	+	+		8	15
		Right	+		+			+		+		+	+					+			7	
	Thalamus and nucleus	Left																				2
		Right							+		+										2	
	Cerebellum	Left																+			1	1
		Right																				
Existence of worms			+				+		+												3	

tion of the cyst, especially the absence of a connective tissue wall, indicated that the worm had but recently reached this location. The surface of the lung was entirely normal. It is evidently impossible that an organism of this size should have penetrated through the surface of the lung from the pleural cavity without producing an obvious break. Therefore the only way in which this case can be explained is by assuming that the worm entered the lung from within, by means of the blood stream. A study of the cerebral sinuses in the dog increased the probability of this course. These sinuses are quite large and situated between the two layers of the dura mater in places of less intimate contact between the cranium and the brain. The cerebral surfaces of the sinuses are made up of a network of loose connective tissue, which would make the entrance of the lung flukes very easy. Once in the sinuses they would be very readily carried along the large internal jugular veins to the heart. This view of the migratory course makes it possible to explain why amongst all the cases in which the lung flukes were introduced into the cranial cavity, both by way of the internal carotid artery and by trephining, only one specimen was recovered inside the cranium, while all the other specimens found were obtained either from the pleural cavity or the lungs. Whenever the worms were found in the pleural cavity the surfaces of the lungs showed the effects of their passage.

IV. AN ANALYSIS OF THE CASES OF CEREBRAL PARAGONIMIASIS REPORTED IN MAN.

In the experiments outlined above we have failed to show how the pathological changes are produced in cerebral paragonimiasis, and what course the parasites take in their migration into the cranial cavity. In Table IV an analysis is presented of the position in the brain of the pathological changes that have been found in the reported cases of human cerebral paragonimiasis, in the hope that some light may thus be shed on these two unsolved questions.

The main pathological changes found in all these cases except that of Musgrave (1907)* are quite similar. They consist of large cysts undergoing necrotic softening in the medullary substance, and some areas of necrobiosis, or of sclerotic foci containing many eggs. These pathological changes are so marked that it seems certain that they must have been produced as the result of invasion on the part of the worms, and by the eggs excreted by the worms themselves during these invasions, and not merely by eggs carried to the brain from elsewhere, as believed by some authors. Adult worms, however, were present only in three of these cases. The extensive pathological changes in those cases where no worms were present can be explained only, if we accept the view that the flukes invaded the brain and then made their way out again. This view is supported very strongly by the evidence brought forward in Section III of this paper which shows a method by which adult worms which have entered the cranial cavity may escape by way of the cerebral sinuses. In some cases where the worms were not found it is possible that they may have died in the unfavorable surroundings of the cranium, and become unrecognizable as the result of post mortem changes. At any rate, it seems impossible that such changes as those which have been described in the human cases of cerebral paragonimiasis, as for example, the foci or pathways containing eggs could have been produced in any other way than by the presence of adult worms in the cranial cavity.

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* Musgrave's description of the pathological findings in his case is as follows: "The membranes of the brain are thickened and adherent in many places; bound in these adhesive masses are cystic, parasitic abscesses similar to those of the pleural cavity. The choroid plexus is coarse and granular, slightly blueish and a few eggs were found in its substance. The brain substance itself appeared to be normal, with the exception of a moderate congestion near the cortical surface. No distinct abscess cavity was found in this organ."

Further, from the table it will be seen that pathological foci in the brain were always situated in the temporal or the occipital lobes, except in Inamoto's case (1915) in which there was only a small parasitic abscess in the left parietal lobe. In every case of cerebral paragonimiasis also some of the pathological foci in the brain were connected with the meninges either directly or through an interposed infiltrated area. From this position of the pathological areas in the brain we can infer that the worms penetrated into the cranial cavity through the foramina situated at the base of the skull. Although there are many apertures at the base of the skull, the jugular foramen seems to be most convenient for the entrance of the parasite, because it is very large and is not completely filled by blood vessels and nerve trunks and through this opening the worms could easily have reached the positions where the pathological changes have been noted in the case records. The finding, by the senior author, in two experimental dogs, also, of partly grown flukes in the neck region, apparently traveling forward in the connective tissue surrounding the blood vessels and nerves which pass through the jugular foramen, is suggestive that this opening is the essential passageway into the cranium.

We may conclude therefore, that the pathological changes in the brain are almost always caused by the entrance of partly grown or adult worms through the foramina at the base of the skull, and their subsequent invasion of the brain tissue, and not by emboli composed of eggs. The exceptional case of Musgrave, however, can only be explained by the supposition that the pathological changes were produced by emboli of eggs, since he found some eggs in the choroid plexus without striking changes in the brain.

Thanks are due to Dr. W. W. Cort of the Department of Medical Zoology of the Johns Hopkins University for his help in revising the manuscript. We also wish to express our thanks to Dr. S. Murai, Assistant Professor of Surgery in the Medical College of Formosa for his aid in performing the operations on the experimental dogs.

Summary.

1. Immature specimens of *P. westermanii* which had been developing for varying periods of time in other animals did not migrate into the cranial cavity of dogs when introduced into wounds in the sides of their necks.

2. Young distomes newly emerged from their cysts did not penetrate into the cranial cavity when introduced into the orbits of dogs, but in same cases made their way into the pleural cavity.

3. Young distomes which had just escaped from their cysts when injected into the common carotid of dogs under conditions which precluded the possibility of their not being carried into the cranial cavity were never found in the cranium, but in several cases were encountered in the pleural cavity.

4. When specimens of *P. westermanii*, both larvae and adults were introduced directly into the cranial cavity of dogs, by trephining the skull, only one out of of over one hundred worms was found in the cranial cavity, but a number were met with in the lungs or pleural cavity.

5. The course which these worms followed from the cranial cavity to the lungs and pleural cavity was probably by way of the cerebral sinuses, the internal jugular veins and the pulmonary arteries.

6. That the cranial cavity is an unfavorable location for the development of *P. westermanii* is suggested by the relative infrequency of cerebral paragonimiasis in man, the finding of a worm in this location showing retardation in development, and by the rapidity with which the worms introduced into the cranium migrated from this location.

7. An analysis of the human cases of cerebral paragonimiasis indicates that in the great majority the pathological changes are produced by the entrance of partly grown or adult worms through the formina at the base of the skull, and their subsequent invasion of the brain tissue, and not by emboli composed of eggs.

BIBLIOGRAPHY.

ANDO, R.
1917. Investigation on Paragonimus westermanii; 5th report (Japanese). Chu Gai Iji shimpo, No. 885.
INAMOTO, K.
1915. Investigation on Paragonimiasis in Korea (Japanese). Chosen Iga Kkai Zasshi, No. 16.
KARASAWA, M.
1909. A contribution to the knowledge of brain tumors (Japanese). Nika gaku Zasshi, No. 127.
KATSURADA, F.
1900. Beitr. z. Kennt. d. Dist. westermanni. Ziegl. Beitr. z. Path. Anat., XXVIII, 506–523.
KAWAMURA, R., ISHIHARA, S., AND YAMAGUCHI, S.
1918. On a new district of Paragonimiasis in Niigata prefecture (Japanese). Tokyoer med. Wochenschr., No. 2064.
KAWAMURA, R., AND YAMAGUCHI, S.
1918. Investigations of Paragonimiasis in Niigata prefecture (Japanese). Hoku yetsu Igakkai Zasshi, XXXIII, No. 6.
KIMURA, D.
1913. A case of cerebral Paragonimiasis in man (Japanese). From a collection of articles in honor of the 25th anniversary of Professor Kure.
MISUMI, J.
1907. Concerning the character of the brain cysts in infection with the lung fluke (Japanese). Chinjei Iho., No. 103.
MUSGRAVE, W. E.
1907. Paragonimiasis in the Philippine Islands. Philippine Journal Sci., Manila, Section B., II, No. 1, 16–63.
OTANI, S.
1888. Nachtrag zum Obduktionsbericht eines Lungendistomen Falles (Japanese). Mit. med. Ges. Tokyo. I, Heft 8–9.
1889. Weitere Mitteilungen über die Lungendistomenkrankheit (Japanese). Mit. med. Ges. Tokyo. II, Heft 6.
1890. Berichtung über das Distoma pulmonale (Japanese). Mit. med. Ges. Tokyo. IV, Heft 24.
1891. Berichtung über das Distoma pulmonale (Japanese). Mit. med. Ges. Tokyo. V, Heft 2.
TANIGUCHI, N.
1904. Ein Fall von Distomerkrankung des Gehirns, mit dem Symptomencomplex von Jackson'scher Epilepsie, von Chorea und Athetosis. Arch. f. Psychiatrie und Nervenkrankheiten, XXXVIII, 101–121.

TSUNODA, G.

1908. Durch Distoma westermanni erzeugte Hirnerkrankung mit dem Symptomencomplex des Hirntumors. Wiener med. Wochenschr., LVIII, Nos. 47–48.

YAMAGIWA, K.

1892. Über die Lungendistomum Krankheit in Japan. Virchow's Archiv, CXXVII, 446–456.

YOKOGAWA, S.

1916. Investigations on the migratory course of the lung fluke in the final host (Japanese). Taiwan Igakkai Zasshi, Nos. 163–164.

1916. Additions to the knowledge of the pathology of the lung fluke (Japanese). Taiwan Igakkai Zasshi, Nos. 163–164.

1916. Investigations on the migratory course of the human lung fluke, Paragonimus westermanii in the final host. Nisshin Igaku, VI, No. 2.

1916. Investigation regarding the development of the lung distome,. especially of a small distome developed from a small encysted cercaria in fresh-water crabs in Formosa. Tokyoer med. Wochensch., Nos. 2030–2031.

1919. Investigations on Paragonimiasis (Japanese). Issued by the Government of Formosa.

1919. Studien über die Übergangs- and Verbreitungswege des Paragonimus westermanii. Verlag der Generalregierung von Formosa.

YOKOGAWA, S., AND SUYEMORI, S.

1917. On the abnormal courses of infection by Paragonimus westermanii (Japanese). Tokyoer med. Wochenschr., No. 2099.

1918. On the migratory course of the lung fluke from the orbits to the pleural cavity, in experimental animals. (Japanese.) Okayama Igakkai Zasshi., No. 354.

1920. Observations on abnormal courses of infection by Paragonimus ringeri. Jour. Parasit., VI., 183–187.

YOKOGAWA, S., SUYEMORI, S., AND MURAI, S.

1919. Experimental investigations on the intracranial parasitism of Paragonimus westermanni. (Japanese). Verhandlungen d. Japanesischen pathologischen Gesellschaft.

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STUDIES ON THE LIFE HISTORY OF VAHLKAMPFIA PATUXENT N. SP., PARASITIC IN THE OYSTER, WITH EXPERIMENTS REGARDING ITS PATHOGENICITY.*

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PLATES IX TO XI.

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INTRODUCTION.

The interest in protozoa has been steadily increasing in the last few years. Many diseases have been found to be caused by various protozoa and still other protozoa are regarded as highly suspicious. Indeed the probable cause of many illnesses not already accounted for is thought by competent research workers to be due to protozoa whose pathogenicity has not yet been determined.

There are many cases reported of persons becoming ill from eating raw oysters. So far the cause of these illnesses has not been determined. It seemed timely therefore, from a public health point of view, to make a protozoan survey of the oyster beds of Maryland with the object of determining what protozoa are found in the oysters and whether they are pathogenic.

Our present knowledge of the protozoan parasites of the oyster is very scant. There is, however, a rather large amount of literature on the spirochaetes inhabiting the stomach and intestine and especially the crystalline lens of the oyster. Certes (1882) was the first person to describe this spirochaete which he called *Trypanosoma balbianii,* to which "Certes" has since been added. He found it in *Astrea edulis* and *Astrea angulata* on the west coast of France. In 1883 Möbius published observations on this same *Trypanosoma balbianii* Certes and said that he had observed it first in 1869 in the oysters of Schleswig Holstein. In the same year (1883) Ryder, working at Woods Hole, notes the presence of these spirochaetes in *Astrea virginica,* and observes that "upon eating these same individuals, known to be infected with parasites, no inconvenience was experienced.

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* From the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University.

showing that these organisms, whatever they may be, are probably harmless to man.'' Since then much has been published on this particular parasite of the oyster.

In 1914 I published an account of the life history of an amoeba parasitic in the digestive tract of the oyster. Unfortunately at the time I could not test its pathogenicity and the strain of amoebae was lost while I was away doing war work.

In the fall of 1919 I began a protozoan survey of the oyster beds of Maryland. Soon after starting this work, I found a rather large amoeba in the digestive tract of the oysters from the Patuxent river. This amoeba was isolated and cultured and forms the basis of the present work.

The purposes of this work are to study the life history of this amoeba, to observe its reactions to different environment, and to test its pathogenicity. So far the study of the life histories of the forms parasitic in invertebrates has proved most helpful in the interpretation of the phenomena observed in the life histories of the amoebae parasitic in man.

METHOD.

In accordance with the rules adopted by the Committee on Standard Methods of Shell Fish Examination of the American Public Health Association, the oysters were first scrubbed and thoroughly washed with running water in order to free them from dirt and from small mussels growing on them. They were then dried in the air and the edges of the shell, where the oyster was to be opened, were flamed in a gas burner. Then the edges were cracked off with a hammer which had been flamed, and a clean scalpel, which had also been flamed, was inserted and the adductor muscle cut, so that it was easy to remove one of the shells.

The liquor of the oyster was then drawn off with sterile pipettes and the oesophagus and later the anus were cut open with sterile scissors. The contents of the stomach and intestine were removed with a fine sterile pipette and streaked out on agar plates. These were kept in moist chambers at room temperature.

CULTURE MEDIA.

Solid media.—As a basis for the solid media 1.5 per cent. agar agar was used. This per cent. of agar had been shown (Hogue, 1917) to be the best for the growth of amoebae. It was usually made up with .7, 1, or 1.5 per cent. sodium chloride solution. The .7 per cent. solution was mostly used as it was kept in stock in the laboratory and the amoebae grew as well on it as on any of the other sodium chloride solutions. In fact all three solutions gave equally good results. More than 1.5 per cent. of sodium chloride was not used with these amoebae, although with *Vahlkampfia calkensi*, also from the oyster (Hogue, 1914), as high as 3 per cent. was used. To all these sodium chloride media .4 per cent. peptone was added. When the bacterial growth became too luxurious, and was killing out the amoebae, the amount of peptone was reduced to .2 per cent. This kept down the rapid growth of the bacteria and gave the amoebae a chance to regain their normal size.

Neutral red was added to some of the sodium chloride agar agar tubes. It was hoped that the bacteria on which the amoebae live would take the stain. This however did not happen.

Agar agar 1.5 per cent. was also made up with Locke solution after the following formula:

Sodium chloride	0.9	gram
Calcium chloride	0.024	gram
Potassium chloride	0.042	gram
Sodium bicarbonate	0.02	gram
Dextrose	0.25	gram

Several attempts were made to grow amoebae on agar agar made up with distilled water to which .4 per cent. peptone was added. The amoebae did not live on this medium.

Liquid media.—Two liquid media were used: Locke solution and ovomucoid. To make the ovomucoid the white of one hen's egg was added to 200 cc. of 1 or 1.5 per cent. sodium chloride solution. This was well shaken up and cooked on a hot water bath for half an hour. It was then filtered through cotton into test tubes. To each test tube was added a loop full of the yolk of the hen's egg (Wherry, 1913). It was then autoclaved.

All the media were sterilized in the autoclave for twenty minutes under fifteen pounds of pressure. The solid media were poured into sterile Petri dishes. Slants were occasionally used, though the amoebae multiply so rapidly that a test tube did not offer a sufficiently large surface for growth and hence required too frequent transplanting to be practicable.

Stab cultures.—Experiments were made with stab cultures. Test tubes with 1.5 per cent. agar, .4 per cent. peptone, and 1, and also 1.5

per cent. sodium chloride solution were inoculated by the stab method with three pure lines. In all cases the amoebae grew luxuriously on the surface of the agar only. The growth of bacteria in the agar was slight and filiform in character.

TECHNIQUE.

A drop of .7 per cent. sodium chloride solution was put on a clean slide and a loop full of amoebae was added. The slide was allowed to remain in a moist chamber from ten minutes to many hours. This gave the amoebae time to attach themselves to the slide and to assume a normal shape before being fixed.

To fix the amoebae the slides were dropped face downward into hot Schaudinn solution and allowed to remain there for about three minutes. They were then removed to the alcohols and stained twenty-four hours in Heidenhain's iron-haematoxylin, .5 per cent. Occasionally safranin was used and with it Licht-grün or acid-violet for cytoplasmic stains. Mann's stain and Rosenbusch's stain both gave very interesting nuclear pictures and brought out many structures which were not clear with Heidenhain's iron-haematoxylin. Dobell's iron haematin was also useful.

Thomson and Thomson (1916) agar slides were used with very helpful results. Melted agar, made up with Locke or .7 per cent. sodium chloride solution, was poured onto a clean slide and then most of it was drained off, leaving only a very thin film of the medium on the slide. This was inoculated with amoebae. The slides were kept in a moist chamber for varying lengths of time and then fixed in cold Schaudinn, stained with iron-haematoxylin, dehydrated and mounted in balsam. It was an uncertain procedure, as the agar with its growth, occasionally floated from the slide and was henceforth useless. Great care had to be taken not to leave the agar too thick, or the oil immersion could not be used, and also not too thin, or it would dry up. The amoebae did not adhere as well to the agar agar as to the glass slide and many were lost in making the preparation.

It was found advantageous to add a loop full of sterile .7 sodium chloride solution to each transplant so as to make the medium a little more moist and so aid the amoebae in spreading out over the plate. When all went well, the results were most satisfactory as you had an exact picture of the amoebae as it was living its life cycle, quite undisturbed by being transferred from the solid medium to the slide or cover slip.

"PURE LINES."

As soon as the amoebae appeared in any considerable numbers on the agar plates, they were transferred to a new Petri dish and allowed to spread out until they were quite far apart. Then, as I described in 1917, a small block of agar agar, containing only one amoeba, was cut out and transferred to a larger piece of sterile agar which was kept in a moist chamber. If these lived and multiplied they were transferred to an agar agar Petri dish. After that subcultures were made with a platinum loop where many amoebae were taken at a time, but as they now were a "pure line," i.e., all derived from one individual, it did not matter how many were transplanted.

BACTERIA.

Bacteria were taken with each transplant. Unfortunately a "pure mixed culture" was not obtained as the amoebae rarely encysted so that cysts could not be obtained in sufficient number to wash them with acids in order to free them from the bacteria. A Barber pipette was not at my disposal when the work was begun so that I could not isolate a single bacterium. However, through the kindness of the bacteriological department I had the bacteria which were growing with one pure line carefully studied and identified. Three different bacteria were present, the prevailing one being a bacillus. None of the bacteria were pathogenic.

GROWTH.

When these sodium chloride media were inoculated with amoebae in the center of the Petri dish, the amoebae spread out quite evenly in ever widening circles over the whole dish, always following the growth of the bacteria. Where the bacterial growth stopped there would be a ring of amoebae five or six deep. Beyond this ring the amoebae were few and widely scattered. The whole Petri dish is usually covered with amoebae in a week's time. Gradually most of these amoebae go to pieces, though a few smaller ones remain scattered about among the debris, often grouped in masses. This is quite different from *Amoeba calkensi* (Hogue, 1914) where many of the amoebae encysted in a week's time.

When the Petri dish is swarming with bacteria, the amoebae do not thrive. They seem to be smothered by the bacteria and the bacterial waste products. Cropper and Drew (1914) say that concentration of the bacterial waste causes amoebae to encyst, but in my cultures, when the bacteria predominate, the amoebae become very small in a day or two, and then usually disappear entirely. Occasionally they emerge along the sides of the bacterial growth and thus work their way out beyond the dense mass of bacteria.

When this balance between amoebae and their food supply is disturbed the best way to bring them again into equilibrium is to transplant them onto Locke agar plates where the bacteria do not multiply so rapidly. Here the amoebae almost at once regain their normal shape and size.

The trophozoites.

1. Movement.

These amoebae have a very characteristic motion. There is a large clear anterior ectoplasmic pseudopodium (Figs. 1 and 9) distinctly marked off from the granular endoplasm. This pseudopodium flows gradually forward, keeping much the same size and shape. Behind it comes the endoplasm. The motion of the ectoplasm is much like that of the sea, as it spreads up on the sand after the waves have broken. At times in certain media, the amoebae send out explosive pseudopodia which go first around one side of the animal and then around the other, like blebs. This, however, is unusual and mostly indicates the setting in of death processes.

2. Morphology.

The amoeba has several characteristic forms. One of the most common of these is a broad fan shaped animal (Fig. 1) having a wide anterior end with a broad flat ectoplasmic pseudopodium, and a pointed posterior end from which usually extend small fine pseudopodia. This form is frequently wider than it is long. When the amoeba is in rapid motion, it elongates and the anterior pseudopodium breaks up into several smaller ones (Fig. 9).

The size of the amoeba varies much according to the age of the culture. For the first three or four days after a Petri dish has been inoculated with amoebae, they are a fairly uniform size, averaging 21μ in length and 13μ in width. After that the size varies greatly and you may find very small amoebae, not measuring more than 1.6μ in length (evidently the result of budding), and giant multinuclear forms (Fig. 6), some of these measuring as much as 143.3μ in length.

The endoplasm is full of small granules and large refractile granules. The large ones never pass out into the ectoplasm. Many of them take the neutral red stain and are probably neutral red granules. Occasionally in the old cultures the small granules pass out into the ectoplasm. This is usually a herald of the setting in of abnormalities leading to death. The ectoplasm disappears and probably mixes in with the endoplasm when the amoeba encysts, and also when it rounds up in response to mechanical stimulation.

Vacuoles.

There is no contractile vacuole, as is to be expected in a salt water protozoon. However there are other vacuoles present. Some of these are food vacuoles and are filled with bacteria and finely granular material. The number of these vacuoles varies greatly. Some amoebae will have none, others will have their endoplasm full of them (Figs. 6 and 11). The size of the vacuoles varies from small spherical ones to a large one practically filling the entire animal and pushing the nucleus off to one side.

The large vacuoles change shape with the changing form of the amoeba. Occasionally when the amoeba is elongating or constricting and pressing against the large vacuole, it will constrict and become divided into two smaller vacuoles. Its contents also divide (Fig. 6). Again two of the vacuoles fuse to make one. Both these processes have been observed in the living amoebae. Still another large vacuole was seen to open and to expel its granular contents to the outside. Unless this had been observed under high power it would have appeared like a contractile vacuole, but the solid content being expelled made this interpretation impossible.

These amoebae have probably taken in more water with their food than they can assimilate and the equilibrium of their cytoplasm has been disturbed, resulting in these numerous vacuoles. The chromatin complex in such amoebae is sometimes disturbed. Figure 11 shows the nucleus broken up into several small masses. This nuclear disturbance does not seem to be fundamental for this amoeba is in the process of dividing.

In the living amoeba these vacuoles with their granular content are often hard to distinguish from endogenous buds. In the stained preparations the difference is clear. The buds (Figs. 2, 5, 9, 10, 12) have a definite outline and a nucleus while the granular contents (Figs. 6, 11) of the vacuoles are irregular in outline and without a nucleus. Some of the vacuoles, especially the smaller ones, are often filled only with clear liquid without granular content. They do not take the glycogen stain with Kofoid's (1919) iodin-eosin formula.

Nucleus.

The nucleus is usually spherical (Figs. 2, 3) and contains a large slightly eccentric karyosome which is also spherical, unless preparing for division or reconstructing after division (Fig. 13*a*). With iron-haematoxylin the karyosome stains a deep blue, though it may contain one or more clear linin spaces (Figs. 6. 9, 13*a*), as has been noted for other amoebae by various investigators. With this stain there is a definite nuclear membrane and often, when the specimens have not been too much destained, the peripheral chromatin appears on the nuclear membrane as small granules (Fig. 2) and occasionally as larger granules (Figs. 5, 31) resembling those of *Entamoeba coli*. Between the nuclear membrane and the karyosome is a clear area.

When safranin and Licht-grün are used the karyosome is brilliant red and the surrounding area a vivid green.

Mann's stain and especially Rosenbusch's stain brought out other details. With Rosenbusch's stain the karyosome appears as a dark blue ring (Fig. 3) with a light grey center. The peripheral chromatin appears on the membrane, while surrounding the karyosome is a light grey area. It appears as a structureless ground substance and of irregular outline, often with fine lines passing out into the clear area which reaches to the nuclear membrane. It appears as some material which is diffusing out from the karyosome. In a recent paper (1920) Huxley has described a " granular band " in the nucleus of *Amoeba clavellinae*. He says at times the band appears structureless and is of varying width. This area seems to closely resemble the light grey area which I have just described for this amoeba. This light grey substance is in the clear area which in the haematoxylin slides fills up the space between the karyosome and the nuclear membrane.

Binary fission.

Division is by promitosis, the karyosome dividing into two parts which form the polar masses. This takes place within the nuclear membrane.

Prophase and metaphase.

The first indication of division is the elongation of the karyosome and with it the stretching out of the nuclear membrane (Fig. 18).

The peripheral chromatin granules soon begin to move around on the nuclear membrane to a point opposite the middle of the karyosome (Fig. 19). Here they become massed together and press against the karyosome so as to indent it. The karyosome thus becomes bean shaped (Fig. 19) and later dumb-bell shaped (Fig. 20), the future polar masses being the ends of the dumb-bell. While this is going on the spindle is forming. Figure 21 shows the fibres of the spindle connecting the chromatin polar masses, in side view. A late stage of this division of the karyosome is seen in front view in Figure 22, where a narrow connecting link is still seen between the polar masses of chromatin.

There is an equatorial plate of chromatin (Figs. 23, 24) which in all probability comes in from the peripheral chromatin. Definite chromosomes were not distinguishable, nor have I been able so far to find later stages in the division of the equatorial plate.

Anaphase.

The anaphase is recognizable by the constriction of the spindle and of the nuclear wall and by the broadening out of the polar masses at right angles to the spindle (Figs. 25, 26).

Telophase.

The constriction of the spindle continues until it is divided into two parts (Fig. 27). Then the nuclear wall divides (Fig. 29) and reconstruction begins at once.

Reconstruction.

The process of reconstruction goes on very rapidly so that it is often difficult to find the stages, even though you know there is much division taking place. But this is true of all the stages in division. The polar masses of chromatin quickly fuse with the chromatin from the equatorial plate, which is always less in quantity (Figs. 13*a*, 28, 30). The spindle material between these two different masses of chromatin passes out to the periphery (Fig. 30). Some chromatin material also goes to the periphery to become the peripheral chromatin. In a few specimens (Figs. 31, 32, 33) one sees fine lines and slender strings of chromatin granules passing from the karyosome out to the periphery. As soon as the chromatin from the old karyosome and from the equatorial plate have fused there is formed an irregular shaped karyosome—often oblong—sometimes pointed as is shown in Fig. 29. Figures for this stage resemble some of those given by Dobell (1919) for *Endolimax nana.* His Figs. 18, 22, 24 and 25 look very much like stages in reconstruction after promitosis. Again the two masses of chromatin may be slow in fusing. In this case the peripheral chromatin is seen far from the chromatin mass, but attached to it by the slender spindle threads (Fig 13*a*). This is found on slides where there are many multinucleate forms and where nuclear division is going on very rapidly. These two masses of chromatin of different sizes might easily be interpreted by the inexperienced investigator as the giving off of chromatin from the nucleus, the small amount coming from the equatorial plate being interpreted as the part which would form chromidia. But this interpretation cannot hold as the later stages show the smaller amount of chromatin being drawn into the larger amount and fusing with it (Fig. 28). Occasionally I found a slide where the majority of the amoebae were in this stage of reconstruction. It always occurs in the young cultures. Sometimes one nucleus will reconstruct much more rapidly than the other, so that there will be two stages of reconstruction in one individual, but this is rare (Fig. 29). In all the stages of division it was very difficult to see the polar cap. Figure 28 shows it in a reconstructing nucleus above the polar mass of chromatin.

Cytoplasmic division.

Division of the nucleus is normally complete before the amoeba begins to divide into two new individuals (though at this time reconstruction may not be complete). During division the amoeba moves about freely. This is contrary to Jepp and Dobell's (1918) observations that "most amoebae when undergoing division are rounded and at rest." When this amoeba is dividing it forms a large pseudopodium at each end (Fig. 17). This helps the newly forming individuals to pull apart. With their pseudopodia they move about, but remain connected for some time by a long slender filament. Finally one individual is stronger than the other, the connecting thread breaks and the two amoebae move apart, as free individuals. On the agar agar slides these newly formed individuals are easily recognized, especially when reconstruction is not entirely complete and the nuclei of the new individuals are in the same stage of reconstruction.

Multiple fission.

Multiple fission was occasionally observed on Petri dish cultures and one slide was made which contained over 350 amoebae in binary fission (Fig. 17), 44 which were dividing into three amoebae (Fig. 15), and many others which were dividing into four, five and even six (Fig. 16) new amoebae. All these dividing amoebae were large and contained numerous nuclei. There were also on this same slide other large multinucleate forms which had not begun to divide. It was a rich culture. The amoebae on the slide were so close together as to be crawling over each other for want of space and there were several masses of amoebae. There were literally thousands of amoebae on this one slide. Nuclear division had gone on more rapidly than the cytoplasmic division and this multiple fission was the means taken to get back to the normal uninucleate state. But even in this multiple fission the newly forming amoebae (Figs. 15, 16) have more than one nucleus so that it would take still another division to reach the normal condition.

This multiple fission has already been noted for limax amoebae by Wherry (1913), Walker and Sellards (1913), Cropper and Drew (1914) and others; and for *Entamoeba histolytica* recently by Yoshida (1920). However none of these observers have figured more than five new amoebae forming at one time.

Multiple nuclear division was found only once (Fig. 7). Here two nuclei side by side are beginning to form spindles in preparation for division. Later stages were not found. Multinucleate amoebae are usually formed by the repeated division of nuclei and not by the simultaneous division of two or more nuclei.

Budding.

Exogenous buds.

There are two kinds of buds, exogenous and endogenous. Both kinds of buds are found in a culture which is only two days old. Here the amoebae are getting an abundance of food, and are growing rapidly. There is much mitosis and many amoebae are dividing by binary fission, but there is still in each amoeba a great excess of chromatin material which has to be gotten rid of in some way. In these cultures the extra nuclei are seen crowded close up against the edge of the amoeba and even bulging out beyond it (Fig. 13*b*). This is the beginning of the formation of the first kind of exogenous bud.

The connection between this bud and the amoeba soon breaks and the bud floats off into the surrounding medium (Fig. 4). Some of the buds have quite a little cytoplasm cut off with them (Fig. 3), others have very little and these latter no doubt soon go to pieces. Those with the larger amount of cytoplasm grow and form some of the small amoebae which are so numerous in the older cultures.

In the cultures which are older, beginning with those which are six days old, a second kind of exogenous bud it found. The extra nuclei pass into the anterior ectoplasmic pseudopodium so that at times it is a solid mass of chromatin. Here they take a deep blue stain with iron haematoxylin (Fig. 8) and a deep red with safranin. They gradually work their way to the sides of the anterior pseudopodium and are then cut off as exogenous buds. Their position at the edge of the ectoplasm and at its junction with the endoplasm insure their getting both ectoplasm and endoplasm.

Endogenous buds.

In the two day old cultures there were numerous endogenous buds forming and quite a few which were already formed. Their nuclei were the extra nuclei which had been formed by the rapid nuclear division already noted in these young cultures.

The endogenous buds form within the endoplasm of the amoeba and are cut off from the main body and come to lie in a vacuole filled with clear liquid. This process is figured for *Vahlkampfia calkensi* (Hogue, 1914). First a crescent shaped vacuole appears and partially surrounds the bud which is forming. This vacuole increases in size and then completely surrounds the bud which is thus cut off from the amoeba and comes to lie in a vacuole within the amoeba. The newly formed bud frequently assumes amoeboid form (Fig. 12) and moves about within its vacuole by amoeboid motion. It gradually gets nearer to the surface of the amoeba (Fig. 2) and then the vacuole breaks to the outside, thus setting free the small amoeba.

Sometimes two amoebae were found in one sac (Fig. 5). It was not determined whether two amoebae were originally cut off into one sac or whether one bud divided into two amoebae. Probably the latter explantion is correct as buds with two nuclei (Fig. 9) were found in one vacuole.

Endogenous buds were found in cultures which were nine days old. After that there is little reproduction of this kind as the amoebae are then quite small. Endogenous buds are evidently another means of getting rid of the extra chromatin material as well as a means of reproduction.

The cycle of reproduction on a Petri dish.

In order to find out whether there was a definite sequence to these different methods of reproduction an experiment was performed in which slides were made every two days from one particular Petri dish culture. On the slides made when the culture was two days old, one noted the unusually large size of the nucleus compared to the amount of cytoplasm. This is one of the first signs that mitosis is going to take place. Here were many multinucleate forms, binary and multiple fission, much mitosis and quite a few endogenous buds, and many exogenous buds of the first type.

By the fourth day there were fewer binucleate and multinucleate forms. The amoebae were reproducing by binary fission and by endogenous buds and a few exogenous buds. On the sixth day the second kind of exogenous buds, those found in the anterior pseudopodia, appeared and were quite numerous. The amoebae were smaller, there was less binary fission and fewer endogenous buds were found. This condition continued until the ninth day. After that endogenous buds were not formed. Little fission was seen after this time though the exogenous buds continued to form along the sides of the amoebae and especially in the anterior pseudopodium. The experiment was discontinued after the fourteenth day as the amoebae were small and very few in number.

Cysts.

In many respects this amoeba is similar to *Vahlkampfia calkensi* which I found in oysters and have already described (Hogue, 1914). *Vahlkampfia calkensi,* however, had very characteristic cysts, of rather uniform size. They were three layers thick and dorsoventrally flattened. These cysts were practically always present in old cultures, where they were easily recognized by their irregular outline and dense appearance.

In the cultures of this amoeba, such cysts were never seen. This was the first thing to make me think I had a different amoeba. These amoebae, when the culture is old, disintegrate, leaving a plate full of débris with here and there a scattered amoeba still active and normal in its appearance. It is these older amoebae which occasionally encyst. As already stated, a Petri dish of .7 per cent. sodium chloride agar agar medium is completely covered with a dense growth of amoebae in a week's time. Cysts, however, do not begin to appear until the culture is at least ten days old, and then only a few cysts are scattered in between the active amoebae. Cysts were also formed when the amoebae were kept at 35° C.

The cysts are spherical (Fig. 14) and vary greatly in size. Those formed on Petri dishes which had been heated varied from 11–18 μ in diameter, those formed in the hanging drop in the thermostat varied from 4–12 μ in diameter. The cyst wall is thin, transparent and regular in outline. It must be slightly mucilaginous as cysts were found with particles adhering to them. Sometimes long streamers of small amoebae, bacteria and débris would remain attached to the cyst wall and wave back and forth in the currents under the cover slip. Inside, only the granular endoplasm is visible and it appears very dense and flocculent. The nucleus is distinct and easily seen. It is much more prominent here than in the active amoeba, where it appears only as a clear area, free from granules. The endoplasm frequently contains one or more vacuoles.

The cyst wall appears to be a semi-permeable membrane. No definite openings were found in it as Wilson (1916) describes for the soil amoeba. When neutral red or Donaldson's stain are added to the medium, the encysted amoebae soon take the red stain and in a short time go to pieces. For this reason it was found advisable not to use these stains which had been used by Kofoid (1919) and others to test the viability of the cysts. For another reason also the stains were not used. There are usually many active amoebae among the cysts. The addition of neutral red or Donaldson's stain made these amoebae round up and look like cysts, the clear ectoplasm distributing itself rather evenly around the spherical mass of endoplasm and giving the appearance of a cyst wall. These amoebae immediately took the stain and went to pieces more quickly than the stained cysts. They had evidently absorbed the water too quickly and then burst. They were often larger than the cysts and not always perfectly spherical. Occasionally they contained two nuclei while only one was ever found in the cysts. In the cysts too, the nucleus was more distinct than in the rounded amoebae.

After the amoeba had gone to pieces the nucleus remained intact for a long time and many of these nuclei would be found floating in the medium. These detached nuclei were present in all old cultures showing that the nuclear membrane is very tough and resistant.

With Donaldson's stain the vacuoles in the endoplasm sometimes stained pink, but never brown like the typical glycogen vacuoles of *Entamoeba histolytica, Entamoeba coli, Endolimax nana* and *Idoamoeba bütschlii.*

Flagellates.

In the early work with these amoebae before pure lines had been isolated, a small flagellate was frequently found with the amoebae in the cultures. I was anxious to see whether it was a stage in the life history of this amoeba. As soon as the pure lines were started, many attempts were made to induce enflagellation.

Schardinger (1899) was the first person to change amoebae into flagellates and then back again into amoebae. He says that temperature and food are the chief factors in the formation of flagellates. Repeating one of his experiments, with slight variations, amoebae were put in hanging drops of .7 per cent. sodium chloride solution on depression slides with hard vaseline rings. They were kept at 34.8° C. Hourly examinations were made one day and the following day they were repeatedly examined, but no flagellates appeared.

This experiment was repeated and the slides kept at 35° for a week. Then they were placed on the desk at room temperature, but no flagellates were formed. In one slide there were a few forms moving with flagellum like motion. However it was found that the .7 per cent. sodium chloride solution had not been sterile and there was the possibility of these forms being a contamination. When the experiment was repeated with freshly sterilized salt solution no flagellates were found.

Next, agar agar slants were made up with 1 and 1.5 per cent. sodium chloride solution. These were inoculated with the amoebae. It was hoped that the water of condensation in the bottom of the tube would induce the amoebae to enflagellate. When the amount of water of condensation was small, sterile .7 per cent. sodium chloride solution was added in small quantities to the test tubes. These slants and the water of condensation were examined for four months in smear preparations and in hanging drops, but flagellates were not found. Instead cysts were forming.

Again amoebae from six different pure lines were put into hanging drops of sterile .7 per cent. sodium chloride solution. These preparations were inverted over depression slides on parallel bars of hard vaseline so as to admit air. They were kept in moist chambers under frequent observation for forty-eight hours. No flagellates appeared. This experiment was repeated, but always with negative results.

Wherry (1913) has described a method by which he could practically always get flagellates from his cultures of soil amoebae. He grew the amoebae in hens' ovomucoid containing a trace of the egg yolk, and found that he could always obtain the flagellates if he took a loopful of the yolk ovomucoid and mixed it with 3 loopfuls of distilled water and placed it in a Barber moist chamber at 22–25° C. Accordingly hens' ovomucoid was made up with 1 and 1.5 per cent. NaCl solution. These tubes were inoculated with amoebae. Eight different pure lines were used. After the cultures were five days old a loopful was transferred to a cover slip and this was placed on parallel bars of hard vaseline over a depression slide in a moist chamber. The slides were under hourly observation for six hours and were again observed on the following morning, but flagellates did not at any time appear. These experiments were tried with many variations, in some cases .7 per cent. sodium chloride solution was added to the loop of yolk ovomucoid, but all efforts failed to give flagellates. The amoebae remained large and active, sending out many pseudopodia.

Still another attempt to obtain flagellates was made. Wilson (1916) says that the addition of distilled water to the medium was sufficient to cause the whole film of amoebae to enflagellate. Accordingly cover slips were prepared with one drop of Locke and one drop of sterile distilled water, also cover slips with one drop of .7 per cent. sodium chloride. Cover slips of Locke and of .7 per cent. sodium chloride solution were also made. These were all inoculated with amoebae taken from Petri dishes of Locke agar medium. Two different pure lines were used. These cover slips were inverted over depression slides on hard vaseline rings and kept in the thermostat at 35°. They were under hourly observation the first day and at frequent intervals on the following four days, but no flagellate forms appeared. The amoeba remained perfectly normal, moving about quite actively. These experiments were repeated the following week but with negative results.

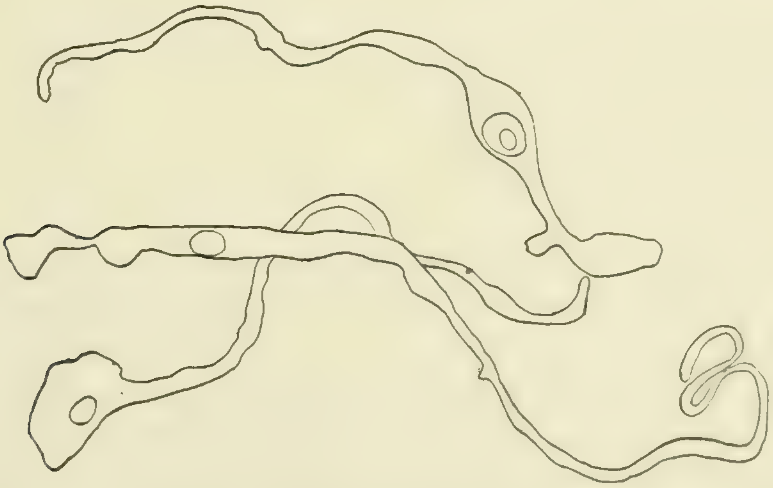
It seems altogether probable that this form of amoeba does not have a flagellate stage, since all attempts to procure flagellates have so far failed.

Age.

The amoebae seem to live indefinitely on Petri dishes of agar agar and .7 per cent. sodium chloride solution with .4 per cent. peptone. One of these cultures has been kept for over fourteen months and it still contains a few small active amoebae. A test tube slant culture of the same medium has active amoebae fifteen months after inoculation. In ovomucoid the amoebae remain large and active after fourteen months growth in this liquid. All these cultures were kept in moist chambers and at room temperature which varied from 18° C. in winter to the summer heat of Baltimore.

Effects of environment.

Media.—I have already shown (Hogue, 1917) that the density of the medium has a distinct effect on the shape of the amoebae. This was further demonstrated when the amoebae were grown on agar slides. If no moisture was added to the agar on the slide the amoebae would be found closely grouped together, fairly overlapping one another. When a loopful of sterile .7 per cent. sodium chloride solution was added to each slide the amoebae moved about freely and those at the edge were found in greatly elongated shapes (Text Fig. 1).



TEXT FIGURE 1. Greatly elongated amoebae grown on agar agar slides to which a drop of .7 per cent. sodium chloride solution has been added.

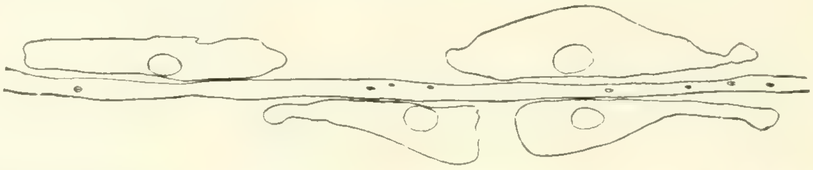
Uhlenhuth has already described (1915) this lengthening out of the cells as being due to the fact that the desired relation between the consistency of the medium and the consistency of the cell plasm has been reached resulting in greater freedom of movement. This difference in the shape of the amoeba has been noted for *Amoeba limax* by Thomson and Thomson (1916).

When neutral red was added to the .7 per cent. sodium chloride agar agar medium the amoebae did not flourish. They grew and in time some of the plates were completely covered with the amoebae, but it was not a luxuriant growth.

On the Locke agar medium the bacteria seem not to have gotten all the food necessary for their rapid growth. Consequently the amoebae multiply much more slowly on this medium than on that made up with sodium chloride, taking twice as long (two weeks at room temperature) to cover the Petri dish. At the end of this time there is an excellent growth. Their shape also is most fantastic, with many pseudopodia extended in search of food.

The amoebae planted on agar made up with distilled water and .4 per cent. peptone did not live. Even the bacteria often died and were never seen to spread over the agar. They were not getting the food necessary for their growth.

Another interesting effect of environment was occasionally seen on the slides which contained a drop of agar medium. Sometimes moulds would get started on these slides. The amoebae growing on the agar would come in contact with hyphae and immediately stretch out along these hyphae (Text Fig. 2). It reminded one of the shape of tissue culture cells when growing along the fibrils of a serum clot.



TEXT FIGURE 2. Amoebae slightly elongated, crawling along a mould hypha.

Liquid media.—Amoebae were planted in test tubes of sterile Locke solution. They lived here for two weeks but did not show any signs of multiplication by budding or by fission. Evidently the bacteria did not thrive and consequently the amoebae did not flourish. The experiment was not followed for longer than two weeks but it was then difficult to find the amoebae in the liquid. They were so small and so few in number.

In ovomucoid the amoebae have lived for over a year without being transplanted into a new medium. They are of excellent size, much larger than the average amoeba grown on .7 per cent. sodium chloride agar medium. The slides made from these cultures show amoebae of all sizes, multinucleate forms, binary fission, and those with endogenous buds. This is certainly one of the best media on which to keep the amoebae for long periods of time. However, they do not multiply as rapidly here as on the solid media. They are well nourished, of good size and nuclear division takes place as is evidenced by the multinucleate forms, but in order to have much fission this amoeba seems to need the contact with a solid medium.

Effects of temperature.

High temperature.—In order to test the effect of high temperature on the cultures a number of Petri dishes containing six different pure lines were put in a moist chamber and kept for twenty-four hours at 35° in the thermostat. The cultures were from two to eleven weeks old. When they were removed from the heat there were very few cysts found, but in a week's time all the cultures had numerous cysts.

Again amoebae from a four day old culture were put in a hanging drop of sterile .7 per cent. sodium chloride solution on a depression slide with a hard vaseline ring. These were kept at 35° C. forty-eight hours. At the end of this time many of the amoebae were rounding up and a few had encysted. There were still many active amoebae and quite a few of them had vacuoles. After 72 hours at 35° still more of the amoebae encysted, though others were still active. On a few slides the drop had spread out around the vaseline ring. On these slides the amoebae were all active and large and no cysts were found. These slides were kept for over a week at 35°. At the end of this time all the slides had some active amoebae and most of them had cysts. The prevailing form was an amoeba, rounded up into a ball, quite inactive but of slightly irregular contour. Under low power it looked like a cyst but with higher magnification it appeared as an amoeba preparing to encyst.

Low temperature.—Several Petri dishes of four pure lines of amoebae were kept on the desk at room temperature, 18–23.5° C. A similar series were placed close to the window in December and January. Here the temperature ranged from 10–15° C. The amoebae divided more slowly in the cold though they were normal in size and shape. When the Petri dishes in the cold were not half covered with amoebae, those at room temperature would be completely covered. At room temperature you found amoebae of all sizes on one dish, while the corresponding culture in the cold had only amoebae of equal size, showing that they had not yet reached the stage of budding. This was due to the slowing down of the life processes at the low temperature. Again in these low temperatures three different pure lines of amoebae arranged themselves in compact groups over the Petri dishes, while at room temperature they were evenly distributed over the plate. Here their locomotion was affected by the cold.

Pathogenicity.

In order to test the pathogenicity of these amoebae it was decided to inoculate kittens with them. Accordingly five kittens were procured and daily microscopical examinations were made of their feces for 7, 7, 8, 8, and 11 days respectively. The feces of each kitten were also cultured on Locke agar. All examinations, both microscopical and cultural, showed no amoebae of any kind in the stools of the kittens.

Since it was very difficult to obtain cysts and since the cyst wall is quite permeable it was decided to inoculate the kittens per rectum. The amoebae from three Petri dishes were scraped off with a platinum loop and put into 3 cc. of sterile .7 per cent. sodium chloride solution. A drop of this was plated out on a Locke agar plate to be sure that the amoebae were in good condition when used for inoculation. All such transplants grew.

The kittens were anaesthetized and each one was inoculated per rectum with 3 cc. of the sodium chloride solution containing the amoebae. They were then given one-half grain of morphine hypodermically so as to slow down peristalsis.

After inoculation daily microscopical and cultural examination of the feces were made for all five kittens for 4, 7, 8, 8, and 11 days respectively. In no case were amoebae recovered. The kittens did not develop dysentery nor did they become infected with the amoebae. Walker and Sellards (1913) say that the amoebae fed in their experiments were recovered from 1–6 days after ingestion, not later, so that these examinations, where the inoculation was per rectum, should surely have shown the amoebae if they had been present in the rectum of the kittens. From these experiments one concludes that the amoebae are not infective to kittens and therefore probably not infective to man. In which case they are non-pathogenic forms.

Systematic position.

In naming this amoeba I am following Hartmann and Schilling's (1917) description for *Vahlkampfia* and putting it in that genus, which was first described by Chatton and Lalung-Bonnaire (1912). For a specific name I propose *patuxent* since the amoeba was found in the oysters from the Patuxent River, near Baltimore.

It can be seen from the description in the preceding pages that *Vahlkampfia patuxent* answers the original description of that genus given by Chatton and Lalung-Bonnaire. They say: "Le nouveau

genre *Vahlkampfia*, comprendra donc les amibes dont le noyau, constitué par un gros caryosome, avec ou sans centriole, peu de chromatine périphérique, se divise par promitose. Elles ont des kystes uninucléés.''

Hartmann and Schilling (1917) give a somewhat fuller description, but with the same essential points. Doflein (1916), however, brings in the presence of a flagellate stage: ''Besonders bemerkenswert ist ihre Fähigkeit in einen begeisselten freischwimmenden Zustand überzugehen.'' Neither Chatton and Lalung-Bonnaire (1912) nor Calkins (1912) nor Hartmann and Schilling (1917) include this stage in their description of Vahlkampfia. I therefore feel justified in placing this amoeba among the *Vahlkampfia,* since, so far, no flagellate stage has been observed in *Vahlkampfia patuxent*. Besides this, its nucleus has a large karyosome without a centriole, and with a small amount of peripheral chromatin. It divides by promitosis, and the cysts are uninucleate; all of which place it in the genus *Vahlkampfia*.

The preceding work is offered as a contribution to the life histories of parasitic amoebae. So far the life history of no parasitic amoebae is completely known. It is hoped that the present work on an amoeba parasitic in an invertebrate, the oyster, will be useful in the interpretation of some of the stages of development of amoebae parasitic in the higher vertebrates and especially of those parasitic in man.

SUMMARY.

1. *Vahlkampfia patuxent* is a non-pathogenic parasite in the digestive tract of the oyster.

2. It has been cultivated in liquid media and on agar agar media made up with various salt solutions.

3. There is a distinct clear ectoplasm and a granular endoplasm.

4. The nucleus is eccentric with a large karyosome.

5. No contractile vacuole is present.

6. Division is promitotic.

7. Division into as many as six new amoebae is figured.

8. Exogenous and endogenous buds are formed.

9. Cysts are of rare occurrence. They are spherical and uninuclear. The cyst wall is thin and slightly mucilaginous.

10. A flagellate stage has not been observed, nor have any of the experiments performed produced flagellates.

11. The amoeba responded to the different media on which it was grown chiefly by a difference in the rate of growth.

12. Low temperature slowed down the rate of division.

13. High temperature caused the amoebae to round up and often to encyst.

14. Kittens inoculated per rectum with these amoebae, did not develop dysentery and were not infected with them.

15. The amoeba is not pathogenic to kittens and is therefore probably not pathogenic to man.

Appendix.

Formal description of *Vahlkampfia patuxent.*

Vahlkampfia patuxent has a clear ectoplasm and a granular endoplasm. There is usually a broad anterior pseudopodium, and several small slender posterior pseudopodia. It varies in size, but averages 21 μ in length and 13 μ in width. The nucleus is spherical with an eccentric spherical karyosome and a small amount of peripheral chromatin on the nuclear membrane. A grey area is visible, with certain stains, around the karyosome.

The nucleus divides by promitosis into two equal daughter nuclei, both the karyosome and peripheral chromatin taking part in the process. Binary and multiple division takes place. There is no contractile vacuole. Endogenous and exogenous buds are formed. The cysts are spherical, 11–18 μ in diameter. The cyst wall is thin, transparent and regular in outline, without openings. No flagellate stage has been found.

It is very similar to *Vahlkampfia calkensi* (Hogue, 1914), but differs from it in having a very simple cyst wall.

Its nuclear phenomena in division are very similar to many of those given by Wilson (1916) for *Naegleria gruberi,* but *Vahlkampfia patuxent* differs from it in being a salt water form without a contractile vacuole and in not having a flagellate stage.

BIBLIOGRAPHY.

Calkins, G. N.

1912. Genera and species of amoeba. Trans. 15th Internat. Cong Hyg. and Demography.

Certes, A.

1882. Note sur les parasites et les commensaux de l'huitre. Bull. Soc. Zool. de France, VII.

Chatton, E. and Lalung-Bonnaire.

1912. Amibe limax (Vahlkampfia n. gen.) dans l'intestin humain. Son importance pour l'interprétation des amibes de culture. Bull. Soc. Path. Exot., V, 135.

Cropper, J. W. and Drew, A. H.
1914. Researches into induced cell reproduction in amoebae. John Howard McFadden Researches, IV.
Dobell, C.
1919. The amoebae living in man. London.
Doflein, F.
1916. Lehrbuch der Protozoenkunde. Jena.
Hartmann, M. and Schilling, C.
1917. Die pathogenen Protozoen. Berlin.
Hogue, M. J.
1914. Studies in the life history of an amoeba of the limax group. Arch. f. Protistenk., XXXV, 154.
Hogue, M. J.
1917. The effect of media of different densities on the shape of amoebae J. Exp. Zool., XXII, 565.
Huxley, Julian S.
1920. Note on an amoeba-like parasite from clavellina. Quart. J. Micro. Sci., LXIV, 413.
Jepps, M. W. and Dobell, C.
1918. Dientamoeba fragilis n. g., n. sp., a new intestinal amoeba from man. Parasitol., X, 352.
Kofoid, C., Kornhauser, S. I. and Swezy, O.
1919. Criterions for distinguishing the endamoeba of amebiasis from other organisms. Arch. Internal Med., XXIV, 35.
Möbius, K.
1883. Trypanosoma balbianii Certes im Krystallstiel schleswig-holsteinischer Austern. Zool. Anzeiger, VI, p. 149.
Ryder, J. A.
1883. The Protozoan parasites of the oyster. Science, I, 568.
Schardinger, F.
1899. Entwicklungskreis einer Amoeba lobosa (Gymnâmoeba): Amoeba gruberi. S. B. kaiserl. Akad. Wiss. Wien. (Math.-Naturwiss. Cl., Abt. I), CVIII, 713.
Thomson, C. D. and Thomson, J. G.
1916. Protozoological researches with conclusions regarding sanitary measures necessary to prevent these diseases. Roy. Army Med. Corps, XXVII, 1.
Uhlenhuth, E.
1915. The form of the epithelial cells in cultures of frog skin, and its relation to the consistency of the medium. J. Exp. Med., XXII, 76.
Walker, E. L. and Sellards, A. W.
1913. Experimental entamoebic dysentery. Philippine Journ. Sci. (B. Trop. Med.), VIII, 253.
Wherry, W. B.
1913. Studies on the biology of an amoeba of the limax group. Arch. f. Protistenk., XXX, 76.
Wilson, C. W.
1916. On the life history of a soil amoeba. Univ. Calif. Press, XVI, 241.

Yoshida, K.
1920. Reproduction in vitro of entamoeba tetragena and entamoeba coli from their cysts. J. Exp. Med., XXXII, 357.

EXPLANATION OF PLATES.

All the figures of *Vahlkampfia patuxent* were drawn with a camera lucida. The plates have been reduced one third.

Plate I.

The drawings were made with 1.9 mm. objective and 7.5 ocular.

Fig. 1. A characteristic form showing broad anterior ectoplasmic pseudopodium and a few slender posterior pseudopodia. The nucleus contains an eccentric karyosome.

Fig. 2. Amoeba with one endogenous bud just ready to break through the thin wall of its vacuole. There are two exogenous buds. The nucleus with its eccentric karyosome shows the peripheral chromatin condensed on one side of the nuclear membrane.

Fig. 3. An exogenous bud being cut off. Mann's stain was used, which brings out the grey area around the karyosome.

Fig. 4. Extra-nuclear bud which has just been given off.

Fig. 5. Amoeba with two endogenous buds in one vacuole. The nucleus shows the peripheral chromatin condensed in plates on the nuclear membrane.

Fig. 6. Giant amoeba with six nuclei. The cytoplasm is full of vacuoles, many containing granular material, which in one vacuole is dividing as the vacuole is constricted by the movement of the amoeba.

Fig. 7. Binucleate amoeba with both nuclei in the early prophase of promitosis.

Fig. 8. Binucleate amoebae with the extra chromatin condensed in the large anterior ectoplasmic pseudopodium. A small exogenous bud is forming at the side.

Fig. 9. Amoeba with a binucleate endogenous bud.

Fig. 10. Amoeba with one endogenous bud formed and eleven exogenous buds forming along the edge.

Fig. 11. Dividing amoeba filled with large vacuoles. The nuclei are abnormal, being broken up into four and five small parts.

Fig. 12. Amoeba with a large endogenous bud which is already moving with amoeboid motion in its vacuole.

Fig. 13*a*. Multinucleate amoeba preparing to divide. Two nuclei are in the process of reconstruction. The equatorial plate chromatin has not yet fused with the chromatin masses.

Plate II.

Fig. 13*b*. A multinucleate amoeba with one exogenous bud cut off and a nucleus near the edge ready to form a second bud. 7.5 ocular, 1.9 mm. objective.

Fig. 14. A cyst showing the prominent nucleus and the endoplasm filled with large refractile granules. 12.5 ocular and 1.9 mm. objective.

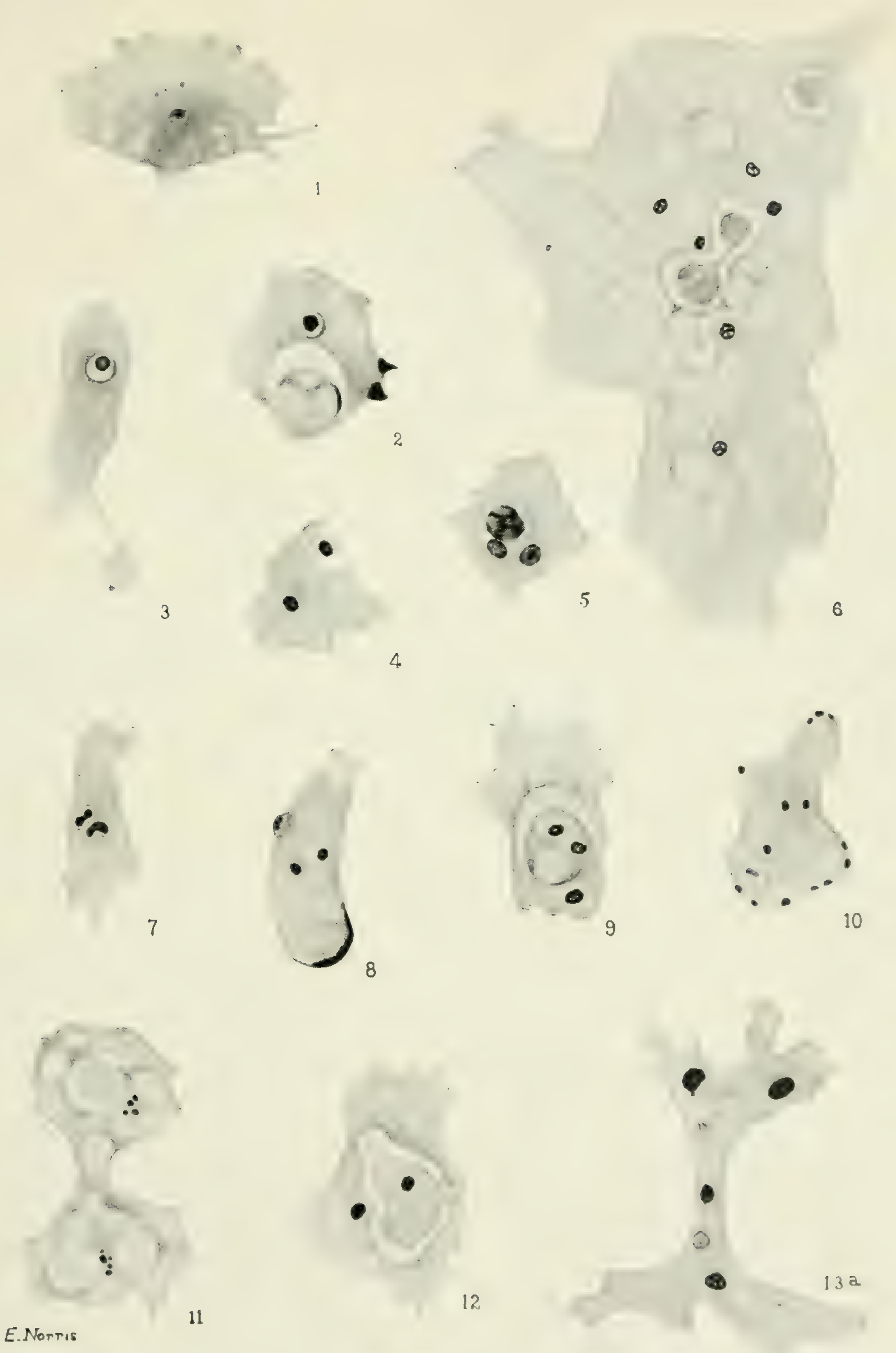


PLATE IX.

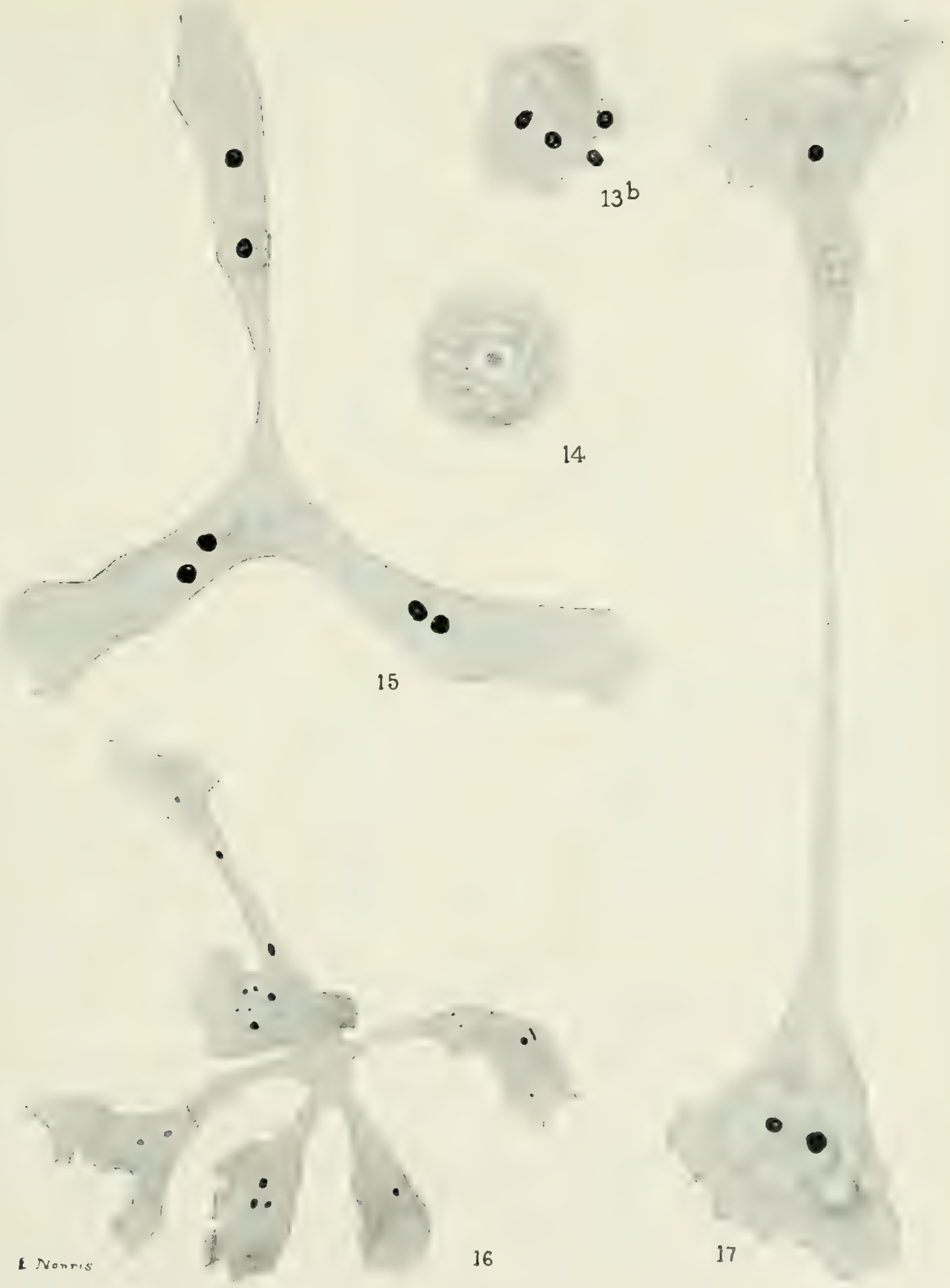


PLATE X.

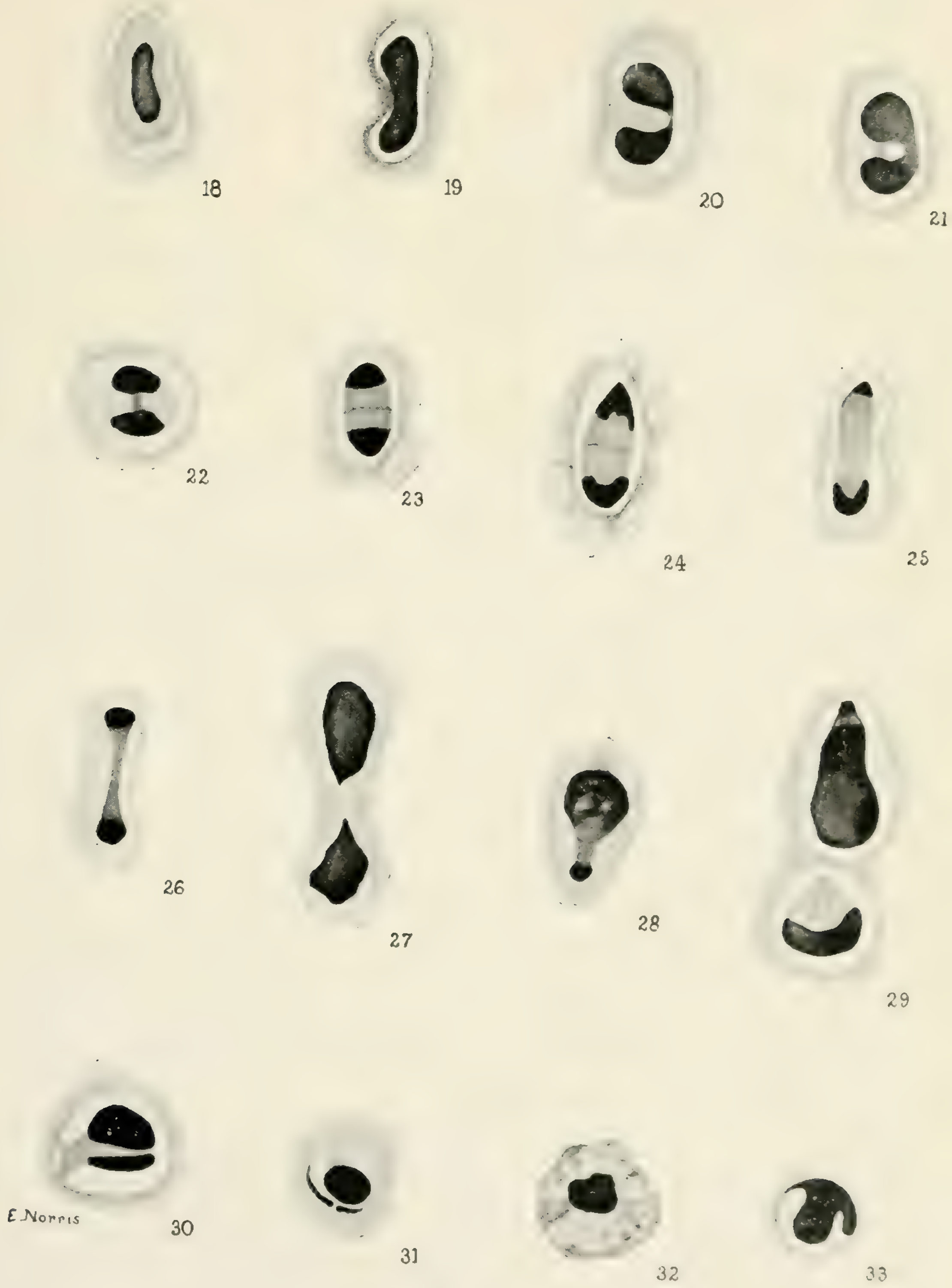


PLATE XI.

Fig. 15. A multinucleate amoeba dividing to form two binucleate amoebae and one amoeba with an endogenous bud. 7.5 ocular and 1.9 mm. objective.

Fig. 16. A multinucleate amoeba dividing to form six new amoebae. 5 ocular, 1.9 mm. objective.

Fig. 17. Amoebae dividing to form two amoebae. 7.5 ocular, 1.9 mm. objective.

Plate III.

All drawings made with 18 ocular and 1.9 mm. objective.

Fig. 18 Prophase: Karyosome, grey area and nuclear membrane all elongating.

Fig. 19. Elongated karyosome being indented by the accumulation of peripheral chromatin at one side.

Fig. 20. Peripheral chromatin dividing the karyosome into the two chromatin masses.

Fig. 21. The spindle fibres showing as a strand between the chromatin masses.

Fig. 22. Formation of the spindle between the chromatin masses which are still connected (front view).

Figs. 23, 24. Late prophase showing broad chromatin masses and equatorial plate.

Fig. 25. Anaphase constriction of the nuclear membrane. Dark areas near the chromatin masses, probably the chromatin from the equatorial plate.

Fig. 26. Late anaphase.

Fig. 27. Telophase. The spindle has divided and the chromatin is distributing itself on the spindle fibers.

Figs. 28–33. Reconstruction.

Fig. 28. Nucleus showing clear polar cap at one end, the chromatin mass and peripheral chromatin connected by the spindle fibers. The clear spaces are plastin.

Fig. 29. Two phases in reconstruction.

Figs. 30, 31, 32, 33. Chromatin material pouring out onto the nuclear membrane where it is condensing.

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GIARDIA (LAMBLIA) INTESTINALIS

A COMMON PROTOZOAN PARASITE OF CHILDREN

By Kenneth F. Maxcy

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Studies of the protozoa found in the intestine of man received a great impetus during the war, due chiefly to the return of large numbers of soldiers from the war area suffering from chronic diarrhœas and dysenteries of various sorts. [166] In investigating the underlying cause of these conditions, a surprisingly high percentage of infestations not only with Entamœba histolytica but also with supposedly non-pathogenic protozoa was disclosed. This led to the examination of non-dysenteric soldiers, of soldiers who had not been outside the country and, finally, of the civilian population in the community at large, with the result that approximately these same percentages were found to hold for all four groups. Pooling the results of all the recent surveys in England, France and the United States, Hegner and Payne[1] find the percentages of infestations with the three commonest intestinal protozoa to be as follows: Entamœba coli 20 per cent, Giardia (Lamblia) intestinalis 12 per cent, and Entamœba histolytica 9 per cent. This work has thrown an entirely different light upon the problem of amœbic dysentery and upon the so-called "flagellate" dysenteries and diarrhœas, of which occasional mention is found in medical literature. It also suggests a reconsideration of the protozoa as intestinal parasites widely distributed in man.

The frequency of Giardia is particularly striking inasmuch as this parasite of man has received little attention in the

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[1] Hegner, R. W., and Payne, G. C.: Scientific Monthly, 1921, XXI 47-52.

[166] United States. Although Lambl first described it in 1859, we are really indebted to Grassi for most of our early knowledge concerning the organism. In the early eighties of the last century, he made a careful study of it and demonstrated its habitat to be the upper intestine, where it might be found in huge numbers perched upon the lining epithelium, to which it adhered by means of a sort of sucking disc. He observed a number of individuals who harbored the parasite and thought that in certain instances it was the cause of diarrhœa.

A little later, 1892, Moritz and Hölzl[2] published a lengthy paper on the infestation of man with Giardia, reporting observations on six patients with diarrhœa and upon finding the organism in the duodenum of ten patients dead of tuberculosis. They noted the frequency with which the parasite occurred in children.

Since these early studies, there have appeared in medical literature a large number of reports of the finding of Giardia in the stools of patients, with or without an accompanying diarrhœa. In the United States attention was first called to it by Stiles[3] in 1902, reporting the case of a patient of Dr. Hemmeter of Baltimore, a three-year-old white child with recurring
[167] attacks of diarrhœa whose stool contained large numbers of the motile organism. In 1910 Allen[4] reported a similar condition in a five-year-old colored girl. Two years later, in an article by Dubois and Toro,[5] we find an account of four children infested with Giardia.

Emerson,[6] in his text-book, mentions a case discovered in the wards of The Johns Hopkins Hospital. In the Public Health Service Reports of 1911, Stiles[7] makes the statement that Giardia is by no means rare in the United States and says that he encountered it in a number of instances in the course of fecal examinations for parasitic worms in North Carolina. Among 6000 admissions to the Mayo clinic, Giardia was found in 66 or a trifle over 1 per cent,[8] whereas among 1000 admissions to Augustana Hospital in Chicago it was recorded in only five instances, or 0.5 per cent.[9] Finally, in a very careful study of 1500 soldiers of the United States Army, 300 of whom had not been overseas, for intestinal parasites, Kofoid, Kornhauser and Plate[10] report 6 per cent infested with Giardia.

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[166] [2] Moritz and Hölzl: Münch. med. Wchnschr., 1892, XXXIX, 831.

[3] Stiles, C. W.: Washington Medical Annals, 1902, I, 64.

[167] [4] Allen, Wm.: Old Dominion Jour. Med. & Surg., 1910, X, 417.

[5] Dubois, E. F., and Wm. C. Toro: Proc. N. Y. Path. Soc., 1912, XII, 32.

[6] Emerson, C. P.: "Clinical Diagnosis," Phila., 2d ed., 1908, pp. 405-406.

[7] Stiles, C. W.: U. S. P. H. S. Reports, 1911, XXVI, 1347.

It will be noted that many authors have regarded this organism as pathogenic—that is, as a cause of diarrhœa. This conclusion is usually based upon the presence in the diarrhœic passages of a large number of motile Giardias which apparently disappear with improvement in the clinical condition. In one or two instances examination of tissues removed at operation or after death has seemed to support the contention. In areas where the surface epithelium had been denuded, these protozoa seemed to have penetrated into the wall of the intestine. On the other hand, many have denied it any pathogenic rôle whatsoever, contending that it was present in diseased conditions of the intestine, not as the primary pathogen, but as a secondary invader, that it deserved no more consideration than is given to Entamœba coli, for instance.

Clinical-pathological studies so far published have, then, failed to solve the problem, and attempts have been made to attack it from the experimental side. The method has consisted mainly in inoculating rabbits,[11] guinea-pigs,[12] and rats,[13, 14] with Giardias obtained from man. This method is complicated by the fact that these rodents are in nature infested with their own species of Giardia: Giardia cuniculi in the rabbit, Giardia muris in culture mice and culture rats, and Giardia microti in the meadow mouse, etc. These species are

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[8] Logan and Sanford: Jour. Lab. & Clin. Med., 1916-17, II, 618-621.

[9] Smithies, Frank: Am. Jour. Med. Sci., 1918, CLVI, 173.

[10] Kofoid, Kornhauser and Plate: Jour. A. M. A., June, 1919, 72: 1721.

[11] Perroncito: Centralblatt f. Bakt., 1887, II, 738.

[12] Stiles: U. S. P. H. S. Reports, Reprint No. 135, 1913, p. 89.

[13] Fantham and Porter: Brit. Med. Jour., 1916, 2900, II, 139-141.

[14] Yakimoff: Compt. rend. Soc. biol., Paris, 1918, LXXX.

[167] more or less similar in appearance to that found in man and can be distinguished only after careful morphological and biometrical study, and observations on the original habitat. If rodents are to be used in inoculation experiments, natural infestation must be carefully excluded (a feat very difficult of accomplishment), and the origin and classification of the strain of Giardia used in the experiment must have been carefully determined. These and other considerations render previous experimental work on the pathogenicity of Giardia inconclusive. For similar reasons, the statements which have crept into the literature regarding rodents as a reservoir of infection for man have not been firmly established.

To recapitulate, it may be said that previous work has demonstrated that Giardia intestinalis is a widely distributed intestinal parasite of man whose power to harm its host has been strongly suspected but is as yet unproved. Assuming that it is capable of harm, then this should be most apparent at the age when the intestinal tract is most sensitive to disturbing influences of all kinds,—namely, in childhood. The literature lends some support to this hypothesis in so far as many of the cases of so-called " Lamblia enteritis " which have been reported have been in children. The problem formulated is, whether Giardia intestinalis is simply a harmless commensal in children or whether it is capable of causing intestinal disturbance.

As a contribution to the solution of this problem the present study was undertaken to determine the frequency of this parasite in the intestinal tract of apparently normal children in the United States. It is obvious that this information is necessary before its presence in those with abnormal intestinal conditions can be properly evaluated.

Methods and Material

Protozoa are apt to be missed in the ordinary clinical laboratory examination of feces. Specimens are rarely examined immediately after passage, although it is known that the actively motile protozoa die and undergo lysis a few hours after they have left the body of the host. Again, the microscopic examination of feces is often carried out with the low power

objective which gives a sufficiently high magnification for the [167]
detection of helminth ova; but the detection of the encysted forms of the protozoa require more careful examination, often of several preparations, and with the use of the higher magnifications.

In pursuing the present study, attention was directed toward demonstrating only the encysted Giardia, and incidentally the other common intestinal protozoa which show encysted forms; *viz.,* Entamœba coli, Entamœba histolytica, Endolimax nana, Chilomastix (Tetramitus) mesnili, Coccidia and Blastocystis.

The specimens were obtained for the most part without the previous administration of a cathartic, and were usually formed stools. Two "wet smear" preparations were made from each specimen, distilled water being used. Material for the one was scraped from the exterior of the stool, any clumps of mucus visible being included; material for the second was taken from the interior. A drop of one per cent water soluble eosin was added to the preparation. The field takes a pink stain; the viable cysts remained unstained and stand out rather
sharply as clearly defined, pale green, refractive ovals against [168]
the pink background. Whenever cysts were found to be present, another preparation was made and stained with iodine (iodine 1 part, potassium iodide 2 parts, distilled water 100 parts). This solution differentiates the structures a little more clearly and brings out the iodophilic inclusions.

About 15 minutes were spent in searching through each preparation before it was discarded as negative. An 18 mm. cover-slip was used, and the field was traversed eight times in each direction, guided by a mechanical stage. The "high dry" objective (4 mm.) with a No. 10 B. & L. eye piece gave an approximate magnification of 450 ×.

When possible each patient was examined on two occasions, at intervals of two or more days. Actually the number of examinations averaged two per case. According to Dobell,[15] Wenyon and O'Connor[16] and others, this technique would

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[15] Medical Research Committee, National Health Insurance, London. 1918, Special Report Series Nos. 7 and 15; 1921, No. 59.

[16] Wenyon and O'Connor, J.: Royal Army Med. Corps, 1917, XXVIII, 1-24.

[168] reveal about two-thirds of the actually existing infestations with intestinal protozoa. This figure probably indicates the percentage of Giardia infestations detected in the present study.

The children examined were miscellaneous medical admissions to the clinic of Dr. John Howland, Harriet Lane Home, Johns Hopkins Hospital. Excepting a small group of "feeding cases," these children were admitted because of acute infectious disease, and were free from gastro-intestinal disturbance. They were fairly representative of the child population of Baltimore with the exception that the negro race was not proportionately represented (only 6 in the 89 were colored).

Results

Of the total of 89 children examined, 18, or 20 per cent, were found to be harboring intestinal protozoa of some type. It is noteworthy that in a group of 15 "feeding cases" under one year of age no infestations were found. The youngest positive case was a little girl 17 months of age whose stools showed large numbers of Entamœba coli cysts. From the second year on there was an increasing number of positive cases with advancing age, so that the group of children from 6 to 12 years old showed a decidedly higher percentage of infestations than the children between one and five.

The relative frequency of the protozoa encountered in this series and the instances of multiple infestations are shown.

Giardia intestinalis (alone)	10 cases
Giardia and Entamœba coli	1
Giardia and Blastocystis	2
Giardia, Hymenolepis nana and Oxyuris vermicularis	1
Entamœba coli (alone)	4
	18

The most interesting fact brought out by this study was the large number of children who harbor Giardia intestinalis. If the infants under one year are excluded, then of the remaining 74 children, 14 were infested with this parasite. In other words one out of every five of the children over one year of age examined harbored Giardia. The raw data indicating the sex and age distribution of these children are given in the [168] accompanying table. The group is in itself too small to admit of conclusive analysis, but may prove of value in connection with other reports of a similar character in the literature.

The youngest instance of Giardia infestation was in a little girl 22 months old. In the group of one to five years of age, 17 per cent showed Giardia, and from 6 to 12 almost 40 per cent were positive.

TABLE 1.—AGE AND SEX DISTRIBUTION OF CHILDREN FOUND TO BE INFESTED WITH GIARDIA (LAMBLIA) INTESTINALIS

Age	Males 58		Females 31		Total 89	
	Non-infested 52	Infested 6	Non-infested 23	Infested 8	Non-infested 75	Infested 14
0— 1	12	0	3	0	15	0
1— 2	10	0	4	1	14	1
2— 3	6	1	5	0	11	1
3— 4	9	0	2	1	11	1
4— 5	5	2	1	2	6	4
5— 6	2	0	3	1	5	1
6— 7	2	0	1	1	3	1
7— 8	0	2	1	1	1	3
8— 9	1	0	0	1	1	1
9—10	1	0	1	0	2	0
10—11	1	0	0	0	1	0
11—12	1	1	2	0	3	1
12—13	2	0	0	0	2	0

The history of each one of these positive cases was obtained. None had suffered from chronic diarrhœa so far as could be learned. One gave a history of a rather severe diarrhœa in the preceding summer, but such a history might be expected in at least one of a group of this size, and no conclusions as to its etiology could be drawn. Three of the children showed a tendency toward increased frequency of defecation—three or four times daily. This may be a significant observation, as it is what one might expect from duodenal irritation.

Case 38, a child five years old, had on admission a diarrhœa. The passages were dark brown in color and contained a large amount of yellowish mucus in which were entangled

[168] myriads of motile Giardia. The condition, however, cleared quickly and after two or three days only the encysted forms could be found in the soft, semi-formed stools, which, however, still contained some mucus at times. No history of repeated attacks of diarrhœa was obtainable in this individual.

The intermittency with which Giardia cysts are discharged was particularly well illustrated by two of the series. In case 19, examinations on January 27, 29 and 31 were negative; on February 3 and 5 large numbers of Giardia were found. In Case 37, the examination on February 24 was positive. No cysts were found on February 27, 29 and March 4. On March 6 large numbers of cysts were again found and were still present at the last examination on March 11.

[169] DISCUSSION

This intermittency emphasizes the necessity of repeated examinations for the establishment of a negative result, in order to judge of the heaviness of the infestation or the permanency of a cure. This has been pointed out by many observers. An attempt has been made to show that Giardia undergoes a regular cycle of cyst production. Porter[17] made daily examinations of a group of soldiers infested with Giardia and estimated the number of cysts passed in 24 hours. The number varied within wide limits, being practically nil on some days and more than 300,000 on other days. She estimated the interval between successive maxima and minima at about 14 days. Boeck,[18] working with the Giardia microti of the meadow mouse, found a similar phenomenon but fixed the interval at seven days. Dobell,[19] on the other hand, finds absolute irregularity in the passage of cysts, suggesting that there may be prolonged periods of complete quiescence during which no cysts can be found.

At all events, it is plainly evident that a single examination or two examinations reveals only a part of those actually parasitized. In the series here reported, while Giardia was actually found in one out of every five children examined, a conservative estimate would place those actually infested at close to one in four. [169]

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[17] Porter, Annie: Lancet, London, 1916, I, 1166-69.

[18] Boeck, Wm. C.: Univ. of Cal. Pub., Berkeley, 1919, XIX, 85.

[19] Dobell, Clifford: Lancet, London, 1916, II, 1053.

Results of British investigations[20] indicate a similar high degree of incidence of Giardia in the intestinal tract of children in that country. Matthews and Smith examined 548 children in the Liverpool Royal Infirmary, and, based upon a single examination per case, found 14 per cent infested with this protozoon. A. H. Campbell found 16 per cent positive in a group of 49 children under 12 years of age in the Children's Hospital, St. Michael's Hill, Bristol.

Muriel M. Nutt examined 128 children in Leeds, England. Among a group of children living in an institution (Workhouse), 48.8 per cent were found to harbor Giardia as compared with 23.9 per cent of the children in the Leeds General Infirmary. The higher incidence among inmates of an institution is a significant fact. In the Sheffield Royal Hospital, this same observer found 15.8 per cent of 57 children examined positive for Giardia.

Most of these figures are based upon a single examination per case. It seems to be clearly established that in England Giardia is a very common intestinal protozoon in children. Our own studies indicate that the same thing is true in the United States.

How soon the infestation is acquired is a question of considerable interest and importance. It has been noted that no cases were found among the 15 infants under one year of age examined. Matthews and Smith found none among the 50 infants which were examined by them. On the other hand, Miss Nutt found no less than 6 of the 25 children under 12 months of age examined by her to be harboring Giardia. The ages were respectively: one at three weeks, one at three months, one at nine months, one at eleven months and two at twelve months. That an infant three weeks old may become infested with Giardia is a startling fact, and indicates how soon the food of children in filthy surroundings becomes con-

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[20] Medical Research Council, London, 1921, Special Report Series, No. 59.

[169] taminated with human fecal material. However, this must certainly be an exceptional case, and infestation during the first year of life is a comparatively rare event.

Sufficient figures are not yet at hand to determine at what year of childhood the maximal incidence is found, but it is not unlikely that it is before the fifth year as is the case with Oxyuris vermicularis. Indeed, these two parasites may be transmitted from child to child in much the same way. Experimental work has demonstrated that Giardia cysts can remain alive outside the human host for only a few hours except under very favorable circumstances as regards moisture and absence of other deleterious influences. If allowed to dry, they perish immediately. An intermediate host has not been shown to exist nor to be necessary. The obvious inference on the basis of our present knowledge is a rather direct transference by fecal contamination of fingers and food. Without doubt it is just one more of the unwelcome guests along with the tubercle bacillus and metazoan parasites which children pick up during the " dirty age."

How long the infestation may last is as yet undetermined. This much is certain: A very much higher incidence is detected in children than in adults. The British figures are very consistent on this point. In the same population groups, the percentage of adults showing Giardia cysts is from one-third to one-fifth that of the children. In this country, as previously mentioned, Kofoid, Kornhauser and Plate found 6 per cent of the healthy, young adult soldiers who had never been outside the United States harboring Giardia. If this figure is compared with the 20 per cent of children found infested in this investigation, the same discrepancy is apparent.

The explanation suggested is that in a certain number of children the infestation is not permanent, and that there is either a heightened immunity or lessened exposure in adult life. The same phenomenon has been noted in the protozoon infestations of certain animals, namely, that the young of the species show a far greater degree of infestation than the adults of the same species. Here the lessened exposure is not so obvious and greater emphasis must be laid upon factors of immunity. These latter may simply be changes in the bacterial flora of the intestine or in food habits. To these views [169] Dobell[20] objects that it has not yet been demonstrated in any particular instance that an individual once infested with Giardia has subsequently been ridded of the parasite. Absence of the cysts in the stools does not necessarily mean that the organism is no longer present in the intestinal tract. Cyst production may be in abeyance in adults. He has himself observed an infected individual over ten years, and although it is frequently impossible to find the organism in the stools the [170] infection is still present and has shown no disappearance from the bowel. Obviously it is just as wrong to generalize from this one case, and until it has been proved that Giardia is as frequent in the intestinal tract of adults as in children, it may be assumed that a certain number of children do get rid of their Giardia infestations.

As regards the pathogenicity of Giardia, in view of the large number of apparently normal children harboring the organism the conclusion is almost forced that in a vast majority of instances it is present merely as a harmless commensal. On the other hand, the fact that, in at least one of the 14 positive cases found in this investigation, there was evidence of an intestinal disturbance which could possibly be attributed to Giardia makes necessary guarded inferences as to its entire harmlessness in children—particularly in very young children.

There seem to be authentic instances in the literature in which individuals harboring this parasite have been subject to recurrent attacks of diarrhœa, during which they passed large quantities of yellowish mucus in which occur the unencysted Giardia in myriads, and in whom no other explanation for the diarrhœa is obvious. As Wenyon and O'Connor[21] point out, it is difficult to explain the large quantity of mucus or the occasion of the attacks and, above all, the crowding of the mucus with flagellates, without assuming that the mucus must be produced by the intestine at the site of the Giardia infection and that the flagellates are directly responsible for its production.

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[21] Wenyon and O'Connor: "Human Intestinal Protozoa in the Near East," Wellcome Bureau of Scientific Research, London, 1917.

[170] Before this parasite is ruled out as of no importance to children, a larger experience is required. Its presence or absence should be recorded in a large series of children suffering from nutritional difficulty, intestinal "indigestion," abnormal frequency of defecation, diarrhœa, etc., and compared with a like group of children, normal in these respects, and living under the same surroundings.

On the basis of this recent knowledge it is evident that the mere finding of a considerable number of Giardia in the stools of a child suffering with diarrhœa and dysentery does not necessarily mean that Giardia is the cause of the condition. In fact it would be strange if a great many such cases were not found. Misdirected efforts at therapy should also be avoided. There is no evidence at the present time that any of the innumerable drugs that have been used are of the slightest value in ridding the patient permanently of his infestation.

CONCLUSIONS

1. Giardia intestinalis is present in the intestinal tract of a large percentage of apparently normal children.

2. It is rarely found before the first year.

3. The percentage of infestations appears to be much higher in childhood than in adult life.

4. The finding of a large number of motile Giardia in a diarrhœa or dysenteric stool is not sufficient evidence to establish the etiologic relationship of this parasite.

5. In certain rare instances the parasite may be responsible for some intestinal disturbance, although this point has not yet been firmly established.

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A CONTRIBUTION OF GENETICS TO THE PRACTICAL BREEDING OF DAIRY CATTLE

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When the writer began his work at the Maine Agricultural Experiment Station some 13 years ago the only genetic concept which played any part in or influenced the actual operations of the animal breeder was that of phaenotypic selection. The slogan and the practice, so far as there was any intelligent practice, was to "breed from the best to get the best." This was perhaps theoretically a sound enough doctrine, but there was lacking one essential to its practical operation with the anticipated degree of success. This lack, as the rapidly advancing knowledge in the field of genetics soon clearly showed, was of any precise criterion as to how the best *genotypes* were to be selected, when the breeder operated on the basis only of a knowledge of *somatic* performance or characters alone. As a matter of fact it was easy to show, as the writer did show, in the case of egg production in fowls,[1] and milk production in cattle,[2] that the phaenotypic performance was an exceedingly unreliable guide to real breeding worth.

The real value of the measure of an animal as a breeder—as a factor in the improvement of a herd or flock—is the kind of progeny which it produces. Confining the discussion to performance in respect of milk or butter-fat production, a cow may have herself a perfectly enormous record of performance but she is worthless as a *breeder* if her progeny are uniformly poor producers, which can in fact be easily shown to be often the case. Again a bull may come from ancestry of the highest performing ability in respect of milk and fat production, and consequently he may as a calf command a price in five figures, but such ancestry has repeatedly been shown to be no guarantee in fact that his progeny will be great performers.

The paramount importance of having a bull of such genotypic constitution as to insure relatively high dairy production in his offspring, if one is to attain success in the breeding of dairy cattle, is obvious. Nearly 10 years ago the writer began the intensive study of the practical problem of scientifically measuring the breeding worth or value of bulls of the dairy breeds, to the end that the farmer and breeder might operate more intelligently and successfully. It appeared evident that the most important thing which a breeder of dairy cattle desires to know is whether the animals which he breeds are transmitting productive qualities to their offspring. This information is particularly desired for the herd bull since his offspring are much more numerous than those of any single cow and he constitutes one-half the heritage given to them. It would appear beyond question that if a bull's daughters are on the average poorer milkers, or poorer in the quality of their milk, than the dams from which they came, then the bull is exercising a harmful effect upon the breed. On the other hand, if a bull's daughters are on the average measurably better than the dams from which they came, in productive qualities, then that bull is exercising a beneficial effect on the breed. Stated specifically, if D_d represents the milk production of a daughter of a given bull, and D_m the milk production of the dam of this daughter, then the measure of this bull's transmitting qualities for milk production in respect of this pair would be:

$$\textit{Sire's transmitting power} = D_d - D_m.$$

If the daughter's production is more than the dam's the sign will be plus. Insofar as one daughter-dam pair is indicative the bull is increasing the production of his daughters and consequently improving the breed. If the daughter's production is less than her dam's the sign will be minus. The bull is consequently insofar detrimental to the breed. By the summation of these plus and minus differences with regard to sign, the amount and sign of this summed quantity gives the measure of what he did for all his daughters, and dividing by the number of daughter-dam pairs gives a measure of the mean or average effect of this sire on the breed.

Being convinced that this reasoning was sound, and that if carried out thoroughly the consequences would be bound in the long run to be of incalculable, but great, practical economic value to mankind, the writer inaugurated in his laboratory at the Maine Experiment Station the project of determining the transmitting power as above defined, of every bull in the Jersey breed recorded up to 1916 as having two or more daughters whose milk-producing qualities were known, as well as the producing abilities of the dam. This project involved a very large amount of labor and was interrupted by the war, but has now been finished and will shortly be published.[3] It is the purpose of the present paper to give a brief account of the scope of the work and some of its chief results.

A prerequisite to the comparison of daughter and dam production was an accurate method of correction for change of milk and fat production with age. Considerable study has been given this problem. I showed in 1914[4] that the equation relating milk flow to age in dairy cattle was of the form

$$y = a + bx + cx^2 + d \log x.$$

Since that time these curves have been worked out in my laboratory for all the principle dairy breeds, chiefly by Miner, Gowen, Patterson, and myself. The curve for annual milk production in the Jersey breed is

$$y = 4586.50 + 307.55x - 12.65x^2 + 2216.62 \log x$$

where y denotes milk production in one year in pounds, and x denotes age in years. How closely this equation agrees with the observed facts is shown in figure 1.

It is obviously impossible to present here anything like the total results of this extensive piece of work. As a sample of the sort of results obtained table 1 is shown. This gives the net average change in butter fat production of daughters as compared with dams. The first column of table 1 gives the place occupied by the bull in the series of bulls. The second column gives the name of the bull. The third column states how many

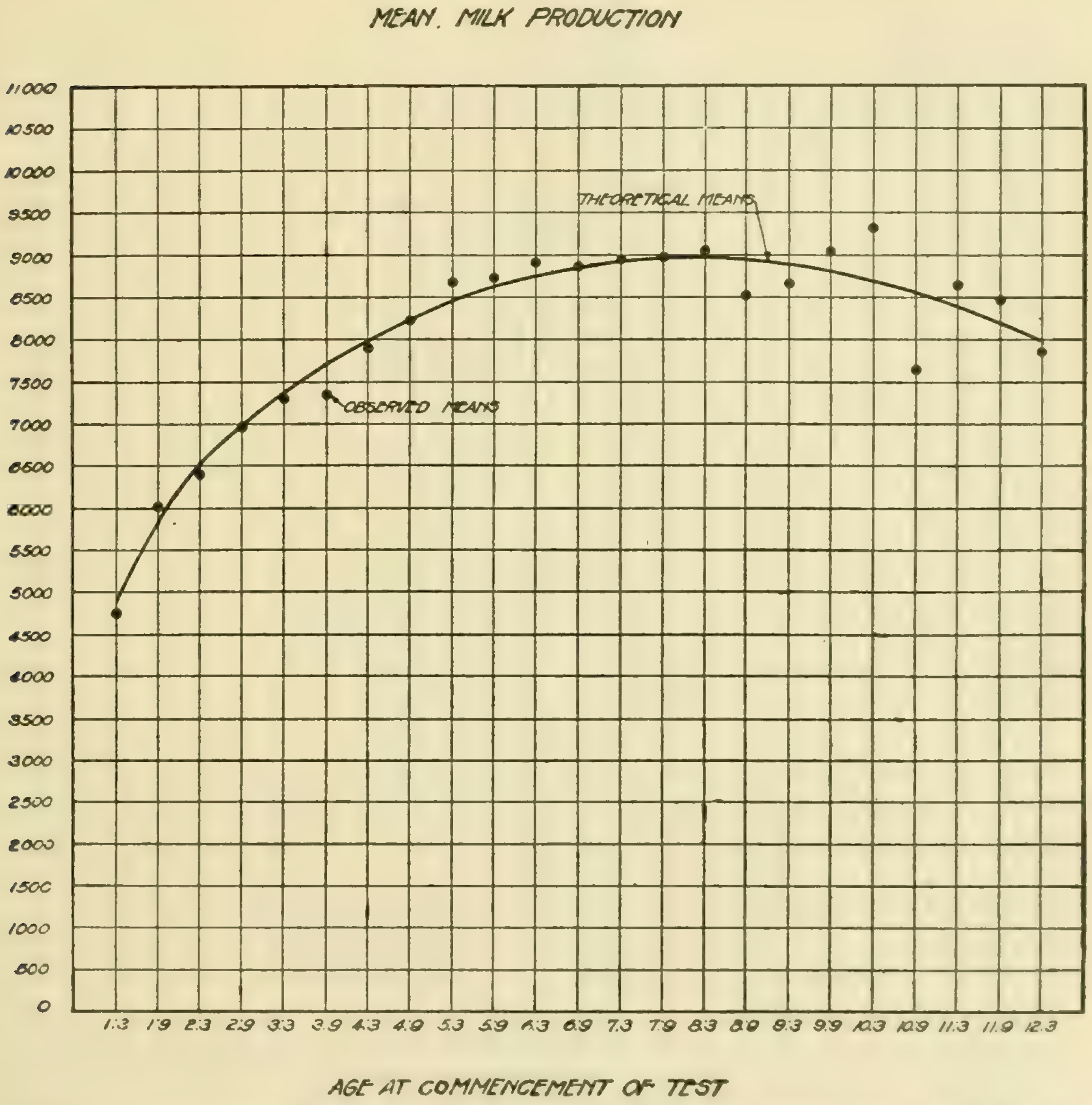

FIG. 1. Observational and theoretical curves of mean milk production (365 days) at various ages in Jersey cows.

pairs of daughter-dam tests the bull under discussion had. The fourth column gives the number of pounds of butter-fat that the bull's daughters are in excess or defect of their dams. It is by this column that the bulls are arranged, the bull whose daughters produced the most butter-fat over the butter-fat production of their dams coming at the top of the list. The plus sign shows the daughters produced more butter-fat than their dams, the minus sign that they produced less butter-fat.

TABLE I

BULLS IN ORDER OF CHANGE IN NET FAT OF DAUGHTERS

NO.	NAME OF BULLS	NO. OF PAIRS	NET CHANGE LBS.
1	Sans Aloi	2	+210.56
2	Signal's Successor	3	+177.72
3	Golden Glow's Chief	4	+161.93
4	Hood Farm Pogis 34th	2	+159.24
5	Tormentor's Landseer Signal	4	+153.55
6	Clear Brook Chief	2	+153.31
7	Channel King	2	+150.30
8	Golden Nero	2	+149.77
9	Majestic Fern	3	+143.97
10	Roma's Rioter of St. L.	3	+143.79
11	You'll Do Oxford	2	+136.61
12	Hood Farm Figgis Torono	3	+132.50
13	Themisia's Interested Prince	2	+131.12
14	Rinda Lad of S. B.	2	+126.38
15	The Plymouth Lad	4	+123.55
16	Hood Farm Torono	34	+121.51
17	Agatha's Brookhill Fox	2	+115.40
18	Fontaine's King	2	+111.60
19	Golden Fern's Son	9	+109.32
20	Baronetti's Golden Lad	2	+100.14
21	Spermfield Owl	26	+97.71
22	Sultan of Oaklands	3	+95.06
23	Melia Ann's King 9th	2	+93.48
24	Chenille's Golden Prince	2	+93.06
25	Eurybia's Blue Boy	3	+90.17
26	Forfarshire's King Dalton	7	+88.00
27	Rioter's Jersey Lad	7	+87.10
28	Queen's Raleigh	3	+82.68
29	Pogis 99th of Hood Farm	14	+82.34
30	Royal Majesty	5	+81.34
31	Jenny's Redfern	2	+79.56
32	Rearguard	3	+79.24
33	Oxford's Fairy Boy	2	+77.14
34	Gertie's Stoke Pogis	3	+70.59
35	Mabel's Raleigh	3	+70.51
36	Pogis 66th of Hood Farm	3	+66.86
37	My Jubilee	2	+66.75
38	Oxford Lass's Cream King	3	+66.23
39	Great Edison	2	+62.78
40	The Warden's Sir Prince	2	+60.87
41	Nova Boy of Turner	2	+58.82
42	Nebraska Lad	2	+57.14
43	Hood Farm Golden Lad	5	+56.50
44	Spermfield Owl 2d	2	+51.85
45	Marston's Interested Prince	9	+51.82
46	Eminent's Tormentor	2	+51.81
47	Chief Engineer	6	+50.20
48	Hood Farm Pogis 9th	39	+47.49
49	The Owl's Double Grandson	5	+47.45
50	Atwell Farm Melia Ann's King	2	+45.84
51	Dorinda Carling's Diploma	6	+41.77
52	Golden Shylock	5	+41.00
53	Loretta D's Champion Son	5	+38.66
54	Taurus Perfection	2	+38.56
55	Brown Bessie's Columbus	3	+38.18
56	Blue Belle's Gold Fern	3	+37.08
57	Golden Lad of Glenwood Farm	3	+35.08
58	Tormentor Summit Lad	4	+34.39
59	Melia Ann's Hero 4th	3	+33.50
60	Keepsake's Golden Lad	5	+32.18
61	Bessie Bate's Lad	2	+30.80
62	Nutley Tones Owl	3	+30.55
63	Flying Fox's Oxford Duke 2d	2	+29.30
64	Lady Letty 4th's Rioter	2	+28.33
65	Golden Lad of Summit	6	+27.79
66	Golden Alcazar	3	+27.32
67	Foxhall's Jubilee	10	+26.64
68	Eminent's Pilot	3	+24.18
69	The Owl of Meridale	5	+24.17
70	G. G. Chief of Ashburn	3	+23.75
71	Imp. Rozel's Noble	2	+22.76

TABLE 1—(*Continued*)

NO.	NAME OF BULLS	NO. OF PAIRS	NET CHANGE LBS.
72	Eminent 4th	5	+21.54
73	Interested Prince	21	+21.07
74	Pogis 75th of Hood Farm	7	+21.00
75	Sayda's Heir 3d	15	+20.87
76	Eminent of Acca Farm	3	+20.39
77	St. Oner's Torono	2	+20.32
78	Flying Fox's Victor	2	+18.58
79	St. Lambert's Ridgewood King	2	+16.32
80	Loretta's King	18	+15.94
81	Romulus of Spring Hill	4	+13.34
82	Cream Princess' Fritz	2	+12.57
83	Irene's King Pogis	16	+12.31
84	Anderson's King	2	+11.11
85	Storrs Golden Lad	2	+9.80
86	The Warden	12	+8.47
87	Lord Letta of Merridale	2	+7.89
88	Eva's Grey Fox	2	+7.67
89	Oonan's Count	8	+7.55
90	Valentines Oonan	10	+5.19
91	Marigold St. Helier	4	+4.42
92	Noble of Oaklands	3	+4.24
93	Fairfield's Chancellor	2	+3.51
94	Exile of the Highlands	4	+3.23
95	Foxy's Brown Poet	7	+2.54
96	Nimbus of Brondale	3	+1.59
97	Gedney Farm Oxford Lad	2	+1.57
98	Foxy's Fox of Althea	2	+1.52
99	Golden Butter Lad	2	+1.25
100	Pedro's Vidi	2	—0.51
101	Mabel's Raleigh	3	—1.46
102	Merry Maiden's Grand son	6	—3.27
103	Model's Oxford Lad	5	—6.99
104	Lookout Torono	17	—7.94
105	Hood Farm Golden Lad 4th	3	—8.37
106	Hazel Fern Golden Kin	8	—8.74
107	Eurybia's Son	3	—9.94
108	Rosetta's Golden Lad	5	—10.04
109	Pogis of Goliad	3	—12.48
110	White Oak Monarch	3	—12.64
111	Prince Noel	4	—13.04
112	Mr. Inez Marigold Pedro	3	—13.49
113	Tonona Pogis	14	—14.00
114	Fontaine's Caiest	5	—15.01
115	Imp. Noble Fontaine Raleigh	5	—15.04
116	Lady Letty's Victor	15	—15.70
117	Guenon's Lad 2d	2	—15.80
118	Oxford's Brigadier	2	—17.48
119	Cinxia's Gamboge Lad	3	—17.69
120	Portland Prospect	3	—18.98
121	Gamboge's Knight	5	—20.07
122	Oxford Daisy's Flying Fox	6	—20.38
123	Hood Farm Torono 21st	4	—20.85
124	Copper Baron	2	—21.24
125	Karnak's Noble	4	—21.25
126	Jersey Lad's Caspar	5	—21.69
127	Marigold's Exile King	8	—22.37
128	Nero's Garfield Lad	2	—22.72
129	Alcarano	4	—23.69
130	Oonan's Major	2	—25.33
131	Merry Miss' Son	5	—26.17
132	Meridale Interested Prince	6	—26.55
133	Oonan's Conqueror	2	—27.48
134	Fontaine's Lodestar	4	—27.56
135	Seven Gates	5	—29.25
136	Noble's Jolly Golden Lad	2	—29.78
137	Naiad's Golden Lad	3	—31.57
138	Romeo of Sheomet	4	—31.75
139	Willson's Exile	2	—33.00
140	Lad's Oxfordshire	2	—33.39
141	Mabel's Poet	5	—36.33
142	Eminent's Raleigh	2	—37.30
143	Reservation Stoke Pogis	5	—37.98
144	Fairy Glen's Raleigh	5	—38.32
145	Gertrude's Jap	2	—38.47
146	Rosaire's Olga Lad	13	—38.93
147	Pogis 95th of Hood Farm	5	—39.71
148	Prince Ramaposa	2	—39.83

TABLE 1—(*Continued*)

NO.	NAME OF BULLS	NO. OF PAIRS	NET CHANGE LBS.
149	Valentine's Count	6	—40.75
150	Sultana's Oxford Lad	3	—40.77
151	Melia Ann's Berkshire	2	—42.39
152	Glory's Pedro	2	—42.85
153	Oxford King of Fernwood	3	—44.37
154	Gertie's Son's Jamont	2	—45.00
155	Pogis Alden Boy	2	—45.04
156	King Allie Rioter	2	—45.11
157	Hood Farm Torono 20th	19	—45.59
158	Olga Hazel Pogis	2	—46.97
159	Marigold's Champion	3	—47.12
160	Sensational Fern	3	—47.58
161	Imp. King of Hambie	2	—48.02
162	Rosaire's Lad of Glenwood	2	—48.79
163	Owl's Model Fox	2	—49.41
164	Rochette's Noble	2	—49.84
165	Biltmore's Torment	4	—50.61
166	Gendey Farm Girl's Oxford	14	—53.27
167	Mistletoe Pogis	2	—53.30
168	Altama's Pogis	3	—53.31
169	Raleigh's Fairy Boy	10	—53.67
170	Quintin Owl	3	—56.07
171	Altama Interst	4	—59.39
172	Golden Lucy's Eminent Lad	7	—60.09
173	Eminent 10th	9	—60.15
174	The Imported Jap	11	—61.91
175	Gamboge's Oxford Lad	2	—64.25
176	Roving Gipsy	2	—64.75
177	Golden Laddie of C.	3	—65.75
178	Lord North	2	—65.98
179	Inez's Stoke Pogis	4	—66.65
180	Golden Paul	3	—68.05
181	Oxford John D.	3	—68.26
182	Ben Hur Pogis	2	—68.79
183	Island Lodestar	6	—70.89
184	Daisy's Prince of St. L.	2	—73.38
185	Pogis 94th of Hood Farm	2	—75.91
186	Amy's Chief	4	—76.37
187	Fontaine's Duke	6	—76.54
188	St. Mawes	2	—78.44
189	Fontaine's Count	2	—80.51
190	Pedro of Brick House Farm	3	—82.31
191	Jacoba's Emanon	9	—84.27
192	Fad	2	—85.61
193	Cowslip's Ashley	3	—87.42
194	Benedictine King	2	—88.14
195	H. F. Golden Fern's Lad	7	—90.49
196	Tormentor's Torono	5	—90.51
197	Girldine's Champion Pogis	3	—90.60
198	Kings Rioter Lawrence	2	—94.96
199	Gazelle's Fawn Rioter King	3	—98.85
200	Noble Peer	4	—98.85
201	Violet's Oakland Count	2	—99.71
202	Minaret Exile	9	—100.59
203	Brown Lassie's Compass	5	—100.61
204	Fort Hill Farm Torono	2	—102.25
205	Viola's Golden Jolly	2	—102.51
206	Oaklands Sultan King	2	—103.95
207	Gertie's Son's Boy	2	—106.18
208	Adelaide's Merry Pogis	3	—108.14
209	Mona Rose's Glory	5	—110.94
210	Financial Raleigh	4	—115.05
211	Jacoba's Premier	3	—115.10
212	Magery Golden's Fox	4	—119.76
213	Fern's Air	2	—123.55
214	Harry B. Gordon	2	—124.46
215	Combination Golden Lad	3	—138.06
216	Hector Marigold	2	—144.12
217	Lady Mary's Fox	2	—148.02
218	Rosaire's Golden Lad	2	—149.95
219	St. Helier of Sheomet	3	—150.59
220	Oonan 23d's Grandson	3	—156.92
221	Jubilee of Bois d'Arc	3	—162.92
222	Oxford Lad's Owl	2	—164.84
223	Hood Farm S. Tormentor	3	—219.72
224	Gertie's Son of Washington	3	—227.01

This table gives to the breeder information of a sort he has never had before. It enables him at once to form a real judgment of the worth of various bulls which appear in the pedigree of Jersey cattle. Many of the bulls in the minus side of the table have at different times been regarded as leaders of the breed and they and their progeny have sold for fabulous prices. With a scientific measure in hand of their actual effect upon the breed it will in the future be exceedingly difficult to persuade any intelligent breeder of the transcendent merits of these minus sires. On the theoretical side there are many interesting points which flow from this and similar tables, but space cannot be taken here for their discussion.

After presenting the basic tables for milk production, fat percentage, and net fat, similar to table 1 here, the complete paper goes on to discuss various related problems, such as the relation of inbreeding, amount of Island bred stock, etc., to production. The chief results of the whole study may be stated as follows:

1. There are 224 Jersey Registry of Merit sires which meet the requirements of this performance test for their transmitting qualities in milk production. One hundred and five of these sires or less than one-half raise the milk production of their daughters over that of the dams of these daughters. The largest number of daughter-dam pairs is 39 for the sire Hood Farm Pogis 9th, 55552. Of those sires which have a large number of pairs, Hood Farm Torono 60326 with 34 pairs stands first in his transmitting qualities raising the milk production of his daughters on the average 2620.1 pounds.

2. Two hundred and twenty-five sires are included in the table giving the sires which met the requirements of the daughter-dam performance test for transmitting qualities of butter-fat percentage. Out of this number 101 sires raised the butter-fat percentage of their daughters' milk as compared with the butter-fat percentage of the dams of these daughters. The leading sire in this butter-fat percentage performance test was Clear Brook Chief 74685 raising his daughters on the average 1.338% of butter-fat. This sire had two daughter-dam pairs. Hood Farm Pogis 9th leads in number of daughter-dam pairs with 42. This bull raised the butter-fat percentage of his daughter on the average of 0.243% over the butter-fat percentage of the dams of their daughters.

3. The sires mentioned as superior in the milk transmitting ability, Hood Farm Torono and Spermfield Owl, do not check up so well in their ability to transmit high butter-fat percentage. Hood Farm Torono caused his daughter on the average to be 0.225 per cent of butter-fat below what the dams of these daughters produced. Spermfield Owl only raised his daughters on the average 0.027% of butter-fat over what the dams of these daughters produced.

4. There are 224 sires of known transmitting ability for net butter-fat. Of this number only 99 sires raise the butter-fat production of their

daughters over that of their dams. The sires which raised the production of their daughters' butter-fat most were Sans Aloi 81012, Signal's Successor 72758, and Golden Glow's Chief 61460. The sires which made the deepest impress on the breed by raising the butter-fat of the largest number of daughters over that of their dams was Hood Farm Torono with 34 pairs and an average increase for each daughter of 121.51 pounds of butter-fat. The next bull, Spermfield Owl, with 26 pairs raised the butter-fat production 97.71 pounds on the average for each of his daughters. Some of the bulls lowering the production of their daughters markedly were Gertie's Son of Washington 83799, Hood Farm S. Tormentor 96311, and Oxford Lad's Owl 75599.

5. The information summarized above was arranged to reveal the transmitting qualities for milk production, butter-fat percentage and butter-fat of Jersey sires to their sons. There were 159 sires which had sons whose progeny performance was known. Of this number 69 or significantly less than half had sons who raised the butter-fat production of their daughters over that of their dams.

6. The sires of superior merit are defined as those which raise the milk production and butter-fat percentage of their daughters as compared with that of their dams. The inferior sires are defined as those sires who lower the milk production and butter-fat percentage of their daughters as compared with the same variables in their dams. The superior sires so defined were arranged by the amount of butter-fat that they increase the production of their daughters over that of their dams. The inferior sires were classified according to the amount of butter-fat that they decrease the production of their daughters in comparison with that of their dams. These two groups of sires are subjected to four generation pedigree analysis to determine their inbreeding and relationship, the amount of Island and American stock, "males and females" and "on the sire's side of the pedigree and on the dam's side of the pedigree," and the individual animals most frequently repeated into two groups of pedigrees.

7. There are 28 sires in the group of sires superior in their transmitting qualities for milk production and butter-fat percentage. In the group of sires inferior in their transmitting ability for these two characters there are 47 sires, a ratio of 1 to 1.7. Such a difference speaks for itself. It emphasizes with startling clearness the need of exact knowledge of the transmitting qualities of bulls to be bred as sires and of the necessity for exact knowledge of the inheritance of milk production and butter-fat percentage.

8. The inbreeding coefficients show that the sires of superior merit have 7.08% of the greatest possible inbreeding up to the fifth generation. The inferior sires are inbred 9.65% of the greatest possible amount (continued brother and sister mating). The group of sires poorer in their transmitting

qualities are consequently more inbred than the group of sires with superior transmitting qualities.

9. The analysis of the pedigrees for the amount of relationship that may exist between the sire and dam of the individual bulls in the superior group and in the inferior group shows that there is little or no difference in the amount of this relationship within the two groups.

10. The resolution of the four generation pedigrees into the Island bred Jerseys and by difference into the American bred Jerseys showed the mean number of Island males in the pedigrees of the superior sires' group to be 8.07 and the mean number of females 7.79. The mean number of Island bred males in the inferior sires' group were shown to be 6.94 and the mean number of females 6.55. The group of sires which increased the production of their daughters over that of their dams had, consequently, more Island bred stock in their pedigrees. The females in each group of the pedigrees had a smaller proportion of Island bred individuals than the males had in each of the groups.

11. Study of the pedigrees of these two groups of sires discloses the fact that all the animals which appeared in the pedigrees of the superior sire on the male side of the pedigrees more than four times or on the female side of the pedigree more than three times also had appearances in the pedigrees of the sires inferior in their transmitting qualities. This fact alone makes it clear that the appearance of certain famous animals in pedigree of a given bull is no guarantee of that particular bull's worth.

My former student and successor at the Maine Experiment Station, Dr. John W. Gowen, expects presently to publish similar investigations for the other chief dairy breeds. It is a great pleasure to acknowledge the faithful and painstaking help of my two assistants, Mr. John Rice Miner and Dr. John W. Gowen in bringing this investigation of the Jersey breed to completion. Without their aid it is doubtful if this would ever have been finished, particularly in view of the influences, which have operated on my own scientific work during the past three years.

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[1] Pearl, R., and Surface, F. M., Studies on the Physiology of Reproduction in the Domestic Fowl II. Data on the Inheritance of Fecundity obtained from the Records of Egg Production of the Daughters of "200-Egg" Hens. *Maine Agric. Expt. Stat. Ann. Rept.*, 1909, pp. 49–84. Also Pearl, R., and Surface, F. M., Is there a Cumulative Effect of Selection? *Zs. Ind. Abst. Ver.*, **2**, 1909 (257–275).

[2] Pearl, R., Breeding for Production in Dairy Cattle in the Light of Recent Advances in the Study of Inheritance, *Eighth Ann. Rept. Comm. Agr. Maine*, 1910, pp. 118–129.

[3] The complete paper will have the following bibliographic status: Pearl, R., Gowen, J. W., and Miner, J. R. Studies in Milk Secretion, VII. Transmitting Qualities of Jersey Sires for Milk Yield, Butter-fat Percentage and Butter Fat, *Maine Agr. Expt. Stat. Ann. Rept. for* 1919, pp. 89–204.

[4] Pearl, R., On the law Relating Milk Flow to Age in Dairy Cattle, *Proc. Soc. Exp. Biol. Med.*, **12**, 1914 (18–19).

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THE EFFECT OF THE WAR ON THE CHIEF FACTORS OF POPULATION CHANGE[1]

THERE have recently appeared some figures[2] regarding the "mouvement de la population" in France, Prussia and Bavaria which appear to deserve somewhat more careful analysis than they have received. These figures are derived from official sources and are conveniently collected in the place to which I have made reference.

There are three factors fundamentally concerned in producing changes in the absolute size of the population in a given fixed area (country, province, etc.). These are:

1. The birth-rate,
2. The death-rate,
3. The net immigration rate.

Of these factors the two first are, generally speaking, of the greatest biological interest. This is particularly true of such political units as France, Prussia and Bavaria, where in normal times net immigration makes no significant contribution to the population. Under war conditions permanent immigration to these units was *nil* and may therefore be safely neglected in the following discussion.

The relation of birth-rate and death-rate changes to population changes is a simple one and may be put this way. If in a given time unit the percentage

$$\frac{100 \text{ Deaths}}{\text{Births}}$$

has a value less than 100, it means that the births exceed the deaths, and that the population is increasing within the specified time unit. If, on the other hand, the percentage is greater than 100 it means that the deaths are more frequent than the births, and that the population is decreasing, again within the specified time unit. The ratio expressed in (i) may be conveniently designated as the vital index of a population.

From the raw data of births and deaths given in the source referred to above I have calculated the percentage which the deaths were of the births for (*a*) the 77 non-invaded departments of France; (*b*) Prussia; and (*c*) Bavaria; and (*d*) England and Wales, from 1913 to 1918 by years, with the results shown in Table I. The English data were obtained from the quarterly returns (No. 284) of the registrar-general.

These percentages are shown graphically in Fig. 1, together with straight lines fitted to each, by the method of least squares. The equations to the straight lines, where y denotes death/birth ratio, and x time, are:

France, $y = 84.0669 + 21.0285\,x$. Origin at 1912. (ii)

Prussia, $y = 59.9 + 17.1\,x$. Origin at 1913, (iii)

Bavaria, $y = 42.4668 + 18.0571\,x$. Origin at 1912. (iv)

England and Wales, $y = 45.9335 + 6.2571\,x$. Origin at 1912. (v)

From this diagram and the data of Table I. we note.

1. In the year prior to the beginning of the war the death-birth ratio of France was at nearly twice as high a level as in any of the other countries dealt with. This fact was of course well known. With a very low birth-rate and a death-rate of the same general order of magnitude as that prevailing in other European countries the French death-birth ratio could not be anything but extremely high.

2. In all the countries here dealt with the death-birth ratio in general rises throughout the war period. This means that the proportion of deaths to births increased as long as the war continued. In France it was

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[1] Papers from the department of biometry and vital statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 14. This paper recently formed the basis of an evening's discussion at the writer's seminar.

[2] *Jour. Soc. Stat. Paris*, Soixantième Année, pp. 356–361, December, 1919.

slightly more than double in 1918 what it was in 1913. The same was in general true of Prussia and Bavaria. These states started from a very different base in 1913, and the relative rise was even greater.

3. In England and Wales, while the death-birth ratio increased throughout the war period, the rate of this increase was markedly slower than in any of the other countries dealt with.

4. A straight line is not a particularly good fit to the French curve, but it has been used in order to demonstrate more clearly the general trend. In 1915 and 1916 the French percentage rose markedly above the straight line. These were perhaps the years when the forces of war impinged most heavily upon the French.

TABLE I

Percentage of Deaths to Births

Year	77 Non-invaded Departments of France	Prussia	Bavaria	England and Wales
1913	97 per cent.	——	58 per cent.	57 per cent.
1914	110 " "	66 per cent.	74 " "	59 " "
1915	169 " "	101 " "	98 " "	69 " "
1916	193 " "	117 " "	131 " "	65 " "
1917	179 " "	140 " "	127 " "	75 " "
1918	198 " "	132[3] " "	146 " "	92 " "

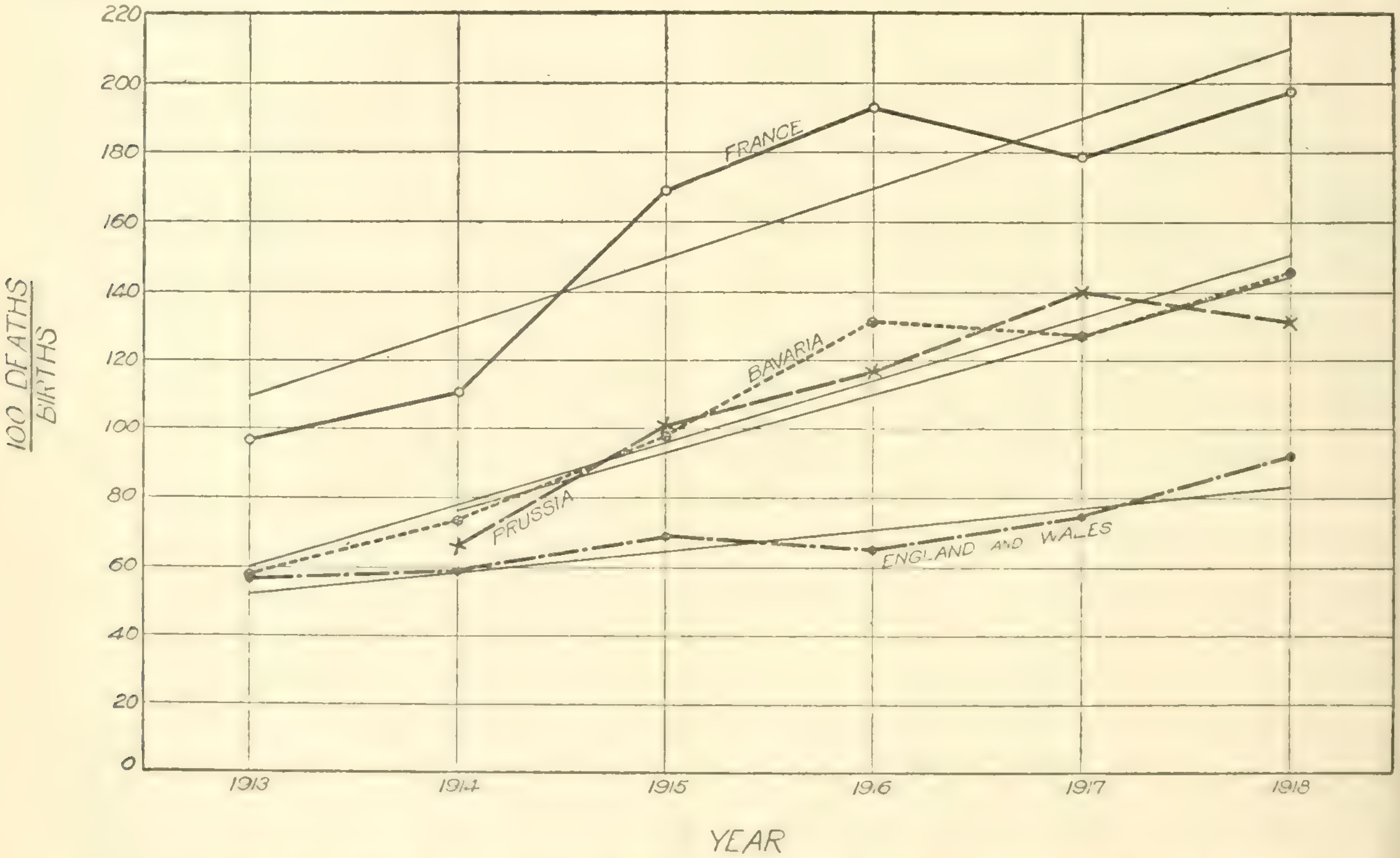

FIG. 1. Showing the change in the percentage, which deaths were of births in each of the years 1913 to 1918 in France (non-invaded departments) (————), Prussia (— —), Bavaria (- - - -) and England and Wales (—-—-).

5. It is noteworthy that despite the epidemic of influenza in 1918, unprecedented in its severity so far as this disease is concerned, none of the curves shows any sharp or marked rise in that year. The curve for England and Wales comes nearest to showing an effect of

[3] This percentage is based upon returns for the first three fourths of the year only.

the epidemic, but even then the rise in 1918 is not so marked as one might have expected.

6. The straight lines for France, Prussia and Bavaria are nearly parallel, or in other words have slopes of about the same order of magnitude (cf., the values of b which determine the slope in the straight line equations). The slope of the line for England and Wales is very different from that of the other three.

These facts raise many interesting points for discussion. The people of Prussia and Bavaria suffered progressive deprivations in respect of food and other comforts of life throughout the war. The sufferings of the French people in these respects were undoubtedly less severe than those of the Germans. All, however, lived for several years on an inadequate diet. This fact alone unquestionably contributed to an ever-increasing death-rate, particularly at the two ends of the life cycle. This same dietary factor undoubtedly also played a considerable part in producing the steady fall in the birth-rate. Here, however, the psychological factor also had a large rôle, and this introduces a point of great interest. Psychologically, the civilian French population and the civilian German population were on a different footing. In the one case, until well into 1918, the attitude was that of the potential conqueror, fighting as an invader in the other's territory. In the other case a war of defense against invasion and further destruction of the home land was being fought. Yet the net effect on the vital indices of the population was, as is shown by the essential parallelism of the straight lines, substantially the same in the one case as in the other. In any other game than war the psychological attitude of offender produces far different results from that of defender. Here the essential and out standing fact is that the net biological outcome of the complex interplay of forces resulting from war was almost identically the same in France and Germany.

Another interesting point is that while France started in 1913 with a death-birth ratio 40 per cent higher than that of the German states—she having at that time an approximate equality of births and deaths—nevertheless the biological changes induced by the war, as expressed in this ratio, were the same for the one as for the others. We are evidently dealing here with deep-seated and fundamental phenomena of racial biology. The biological reactions of French and Germans in respect of a most fundamental phenomenon, the death-birth ratio, were essentially the same, though they started from such different pre-war bases.

The case of England is obviously entirely different. Starting from about the same base as the German states England's biological reaction to war was much less pronounced. There are many explanations, such as better food conditions, different race psychology from any of the other belligerents, etc., which might be brought forward. There appears at the moment no way of accurately evaluating any of these possible explanations. We must perforce rest with the setting forth of the facts. It is worth noting, however, that though England's vital index changed less in degree than that of the other countries, its movement was the same in kind.

There are two other points which one would like to have information upon. The first is: What will be the course of these death-birth ratio curves in the years following 1918? Will they come back to the pre-war level, and if so, how soon? For England and Wales alone is it now possible to get an indication on this point. For the year 1919 the relation $\frac{100 \text{ Deaths}}{\text{Births}}$ had the value 73 per cent. This represents a marked drop, though it does not bring the curve back to the pre-war level. The appearance of official statistics which will make possible the further plotting of the curves of Fig. 1 will be awaited with great interest. In the second place, one would like to know what the appearance of the curve for the United States would be. Unfortunately, we have for the Registration Area of births data only for the years 1915, 1916, and 1917 now available. So few years appear inadequate to set against the longer series for the other countries.

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SOME LANDMARKS IN THE HISTORY OF VITAL STATISTICS*

In the earlier volumes of the Journal of the Royal Statistical Society—those mines of curious information—a favorite form of contribution was the "tabular résumé," which presented a series of more or less statistical facts on a chronological base. With so distinguished a precedent it has seemed to me that the Quarterly Publications might care to present to its readers a small contribution in the same form, which I have recently drawn up for the use of my students in vital statistics.

This "tabular résumé" attempts to set forth in chronological array what seem to me to be some of the most important landmarks in the history of biostatistics. To disarm in some measure criticisms, which from the standpoint of the professional historian would otherwise be undoubtedly merited, I hasten to say, first, that there has been no slightest thought of encompassing within this short table a complete history of the subject. Historical completeness and the tabular form of presentation do not go well together. The object of the present table is much simpler. It is to get before the student the briefest conspectus of the time relations of the development of the subject on the one hand, and of the personalities concerned in a large path-breaking way in this development, on the other hand. The precise manner in which such an object will be carried out will obviously be different for each person who attempts it. One person's estimate as to the relative historical significance of a particular event or personality will differ from another's. In presenting the matter to my class I endeavor to justify in more detail than is possible in the table itself the particular items which appear. In any event, it seems clear that any historical review of vital statistics would be bound to contain at least a good many of the items of the present table. More than this in the way of agreement among scholars on a historical matter it is doubtless idle to hope for.

In the second place it should be said that if the sources chosen for statement of reference as to the fact are obviously in many cases second-hand, and perhaps somewhat casual, this is so of deliberate purpose. I am hopeful that by so choosing them I may perchance entice an unwary student or so to do a little reading about the men who have helped to develop modern statistics. I am quite sure that this will not happen if I refer him straight off to a ponderous and deadly "Geschichte der Statistik." Nor is there much chance that the embryo health-officer would make anything but very heavy weather if he essayed a voyage into the "*Theorie analytique*." But if he will read the article in the Encyclopedia Britannica on Laplace he will tend to have a measure of wholesome respect for a great man and will know a little at least of what that man meant in the history of science.

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* Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health Johns Hopkins University, No. 11.

TABULAR REVIEW OF SOME OF THE IMPORTANT EVENTS IN THE HISTORY OF VITAL STATISTICS

Year	Event	Personality Concerned	Authority for Record
1532	First definitely known compilation of weekly bills of mortality in London	———	Hull, C. H., Econ. Writ. of Sir W. Petty, p. lxxxi
1539	Beginning of official registration of baptisms, marriages and deaths in France	———	Faure, F. Hist. Stat. p. 242
1608	Beginning of oldest parish register in Sweden		Arosonius, E. Hist. Stat. p. 537
1662	Publication of first edition of "Natural and Political Observations Mentioned in a following Index, and made upon the Bills of Mortality"	Capt. John Graunt, Citizen of London (1620–1674)	Hull, C. H. Econ. Writ. of Sir Wm. Petty, p. 315
1666	First Census of Canada (The earliest modern census of population)		Godfrey, E. H. Hist. Stat. p. 179
1669	Application of mathematical theory of probability to expectation of human life	Christiaan Huygens (1629–1695)	Stuart, C. A. V. Hist. Stat. p. 430
1693	Publication of "Estimate of the Degrees of Mortality of Mankind," in the Philosophical Transactions of the Royal Society	Halley, the astronomer (1656–1742)	Hull, *Loc cit.* p. lxxvii
1713	Publication of "Physico-theology; or a demonstration of the Being and Attributes of God from his Works of Creation"	Rev. William Derham (1657–1735)	Hull, *Loc. cit.* pp. lxxvii and lxxviii
1718	Publication of the "Doctrine of Chances"	A. DeMoivre (1667–1754)	Art. DeMoivre, Encyc. Brit
1735	Registration of vital statistics begun in Norway		Kiaer, A. N. Hist. Stat. p. 447
1741	Publication of "Die göttliche Ordnung in den Veränderungen des menschlichen Geschlechts aus der Geburt, dem Tode und der Fortpflanzung desselben erwiesen, etc."	Johann Peter Süssmilch (1707–1767)	Hull, *Loc. cit. p.* lxxviii
1746	Publication of the first French tables of mortality under the title "Essai, sur les probabilités de la durée de la vie humaine"	Deparcieux	Faure, F. *Loc. cit.* p. 265
1748	Beginning of Swedish official vital statistics	———	Arosonius, E. Hist. Stat. p. 540
1749	First complete Census of Sweden	———	Rossiter, W. S. Cent. Pop. Growth, p. 2
1753	First Census of population in Austria ordered	———	Meyer, R., Hist. Stat. p. 85
1769	First population Census of Denmark and Norway	———	Jensen, A. Hist. Stat. p. 201
1790	First federal Census of the United States		
1795	First Census of the Netherlands	———	Stuart, C. A. V. Hist. Stat. p. 43
1797	Establishment of Danish-Norwegian Tabulating Office	———	Jensen, A. *Loc. cit.* p. 201
1798	First complete Census of Spain	———	Rossiter, W. S. Cent. Pop. Growth, p. 2
1801	First complete Census of Great Britain	———	Rossiter, W. S. *Loc. cit.*
1801	First complete Census of France	———	Rossiter, W. S. *Loc. cit.*
1805	Formation of first statistical state office within boundaries of German Empire	———	Würzburger, E. Hist. Stat. p. 3
1810	First complete Census of Prussia		Rossiter, W. S. *Loc. cit.*
1812	Publication of "Theorie analytique des probabilités"	Pierre Simon Laplace (1749–1827)	Encyc. Brit. Art. Laplace
1812	Inauguration of civil registration of births, marriages and deaths in the Netherlands	———	Stuart, C. A. V. Hist. Stat. p. 432
1812 1826	Publication of "Theoria combinationis observationum erroribus minimis obnoxia" (Least squares)	Karl Friedrich Gauss (1777–1855)	Encyc. Brit. Art. Gauss
1815	First complete Census of Norway	———	Rossiter, W. S. *Loc. cit.*
1815	First complete Census of Saxony	———	Rossiter, W. S. *Loc. cit.*
1816	First complete Census of Baden	———	Rossiter, W. S. *Loc. cit.*
1818	First complete Census of Austria	———	Rossiter, W. S. *Loc. cit.*
1818	First complete Census of Bavaria	———	Rossiter, W. S. *Loc. cit.*
1825	Publication of "Mémoire sur les lois des naissances et de la mortalité à Bruxelles," Quetelet's first statistical paper	Lambert Adolph Jacques Quetelet (1796–1874)	Lottin, Quetelet, p. xx
1826	Establishment of statistical commission in Belgium	Ed. Smits	Julin, A. Hist. Stat. p. 126
1829	First official Census of Belgium	Ed. Smits	Julin, A. Hist. Stat. p. 128

TABULAR REVIEW OF SOME OF THE IMPORTANT EVENTS IN THE HISTORY OF VITAL STATISTICS.—*Concluded*

Year	Event	Personality Concerned	Authority for Record
1832	Publication of "Recherches sur la reproduction et sur la mortalité de l'homme aux different ages et sur la population la Belgique d'apres la recensement de 1829 (premier recueil officiel des documents statistiques)"	Quetelet and Smits	Lottin, *Loc. cit.* p. xxi.
1834	Royal Statistical Society (London) founded	———	Title page of Journal
1835	Publication of "Sur l'homme et le dévéloppement de ses facultés, ou Essai de physique sociale"	Lambert AdolpheJacques Quetelet (1796–1874)	Lottin, *Loc. cit.* p. xxi
1836	First complete Census of Greece	———	Rossiter, W. S. *Loc. cit.*
1837	Civil registration of vital statistics in England. Establishment of office of Registrar-General	———	Baines, A. Hist. Stat. p. 370
1837	Appointment of William Farr as compiler of abstracts in the Registrar-General's Office	William Farr (1807–1883)	Encyc. Brit. Art. Farr.
1838	Publication of "Essay on Probabilities" in Lardner's Encyclopedia	Augustus DeMorgan (1806–1871)	Encyc. Brit. Art. DeMorgan
1839	Organization of American Statistical Association		Hist. of Stat. p. 3
1846	Publication of "Analyse mathematique sur les probabilités des erreurs de situation d'un point." Acad. des Sci. Mem. par div. sav. IIe. Ser. t. ix (Correlation)	A. Bravais	Yule Introd. p. 188
1848	Foundation of the Institute of Actuaries of Great Britain and Ireland		Encyc. Brit. Art. "Actuary"
1860	First complete Census of Switzerland		Rossiter, W. S. *Loc. cit.*
1861	First complete Census of Italy		Rossiter, W. S. *Loc. cit.*
1863	Austria establishes Central Statistical Commission	Count Mercandin	Meyer, R. *Loc. cit.* p.89
1865	Publication of "History of Mathematical Theory of Probability from the Time of Pascal to that of Lagrange"	Isaac Todhunter (1820–1884)	Encyc. Brit. Art. Todhunter
1867	First creation of independent official statistical organization in Hungary		Buday, L. von. Hist Stat. p. 395
1869	Publication of "Hereditary Genius"	Sir Francis Galton (1822–1907)	Art. Galton Encyc. Brit.
1869	Foundation of Société de statistique de Paris		Title page of Journal
1872	Opening of German Imperial Statistical Office		Würzburger, E. Hist. Stat. p. 337
1881	First general Census of India		Baines, A. Hist. Stat. p. 421
1887	Royal Statistical Society incorporated by Royal Charter		Title page of Journal
1890	First Census in which mechanical methods of tabulation were used	John S. Billings and Herman Hollerith	Rept. Supt. Census 1889, p. 8
1894	Publication of first of "Contributions to the Mathematical Theory of Evolution" in Phil. Trans. Roy. Soc.	Karl Pearson	Title page
1897	Publication of paper "On the Theory of Correlation" in the Jour. Roy. Stat. Soc.	G. Udny Yule	Jour. Roy. Stat. Soc. Vol. LX, p. 812
1897	First Census of Russia	———	Kaufman, A. Hist. Stat. p. 481
1900	First year of separately published official mortality statistics for Registration Area of United States		Title page of "Mortality Statistics"
1901	Publication of first number of Biometrika	Francis Galton Karl Pearson W. F. R. Weldon C. B. Davenport	Title page
1902	Creation of permanent Census Bureau in the United States	———	Cummings, J. Hist. Stat. p. 682
1915	First year of separately published official birth statistics for Registration Area of United States	———	Title page of "Birth Statistics"

Expanded Titles of References Cited in Table

Encyclopedia Britannica. Eleventh Edition.

Hull, C. H. The Economic Writings of Sir William Petty, etc. Cambridge, University Press, 1899. 2 vols.

Lottin, J. Quetelet, Statisticien et Sociologue. Louvain and Paris. 1912.

Rossiter, W. S. A Century of Population Growth from the First Census of the United States to the Twelfth 1790–1900. Washington, Govt. Printing Office, 1909.

The History of Statistics, Their Development and Progress in Many Countries. Collected and edited by John Koren. New York (Macmillan). 1918.

Yule, G. U. Introduction to the Theory of Statistics. Fifth Edition. London (Griffin & Company). 1919.

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ON THE RATE OF GROWTH OF THE POPULATION OF THE UNITED STATES SINCE 1790 AND ITS MATHEMATICAL REPRESENTATION[1]

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It is obviously possible in any country or community of reasonable size to determine an empirical equation, by ordinary methods of curve fitting, which will describe the normal rate of population growth. Such a determination will not necessarily give any inkling whatever as to the underlying organic laws of population growth in a particular community. It will simply give a rather exact empirical statement of the nature of the changes which have occurred in the past. No process of empirically graduating raw data with a curve can in and of itself demonstrate the fundamental law which causes the occurring change.[2] In spite of the fact that such mathematical expressions of population growth are purely empirical, they have a distinct and considerable usefulness. This usefulness arises out of the fact that actual counts of population by census methods are made at only relatively infrequent intervals, usually 10 years and practically never oftener than 5 years. For many statistical purposes, it is necessary to have as accurate an estimate as possible of the population in inter-censal years. This applies not only to the years following that on which the last census was taken, but also to the inter-censal years lying between prior censuses. For purposes of practical statistics it is highly important to have these inter-censal estimates of population as accurate as possible, particularly for the use of the vital statistician, who must have these figures for the calculation of annual death rates, birth rates, and the like.

The usual method followed by census offices in determining the population in inter-censal years is of one or the other of two sorts, namely, by arithmetic progression or geometric progression. These methods assume that for any given short period of time the population is increasing either in arithmetic or geometric ratio. Neither of these assumptions is ever absolutely accurate even for short intervals of time, and both are grossly inaccurate for the United States, at least, for any considerable period of time. What actually happens is that following any census estimates are made by one or another of these methods of the population for each year up to the next census, on the basis of data given by the last two censuses only. When that next census has been made, the previous estimates of the inter-censal years are corrected and adjusted on the basis of the facts brought out at that census period.

Obviously the best general method of estimating population in inter-censal years is that of fitting an appropriate curve to *all* the available data, and extrapolating for years beyond the last census, and reading off from the curve values for inter-censal years falling between earlier censuses. The methods of arithmetic or geometric progression use only two census counts at the most. Fitting a curve to all the known data regarding population by the method of least squares must obviously give a much sounder and more accurate result. In making this statement, one realizes perfectly, of course, the dangers of extrapolation. These dangers have been well emphasized by Perrin,[3] who used higher order parabolas to predict the future population of Buenos Aires. In keeping sharply before our minds the dangers of extrapolation from a curve, we are apt to forget that the methods of extrapolation by arithmetic or geometric progression have much less general validity than from a curve, and the inaccuracies are found in practice, except by the rarest of accidents, to be actually greater.

The first one to attempt an adequate mathematical representation of the normal rate of growth of the population of the United States was Pritchett.[4] Taking the census data from 1790 to 1880, inclusive, Pritchett fitted by the method of least squares the following equation:

$$P = A + Bt + Ct^2 + Dt^3 \qquad \text{(i)}$$

where P represents the population and t the time from some assumed epoch. As a matter of fact, Pritchett took the origin of the curve at 1840, practically the center of the series. With this third-order parabola Pritchett got a very accurate representation of the population between the dates covered. As will presently appear this curve did not give, even within the period covered, as accurate results as a more adequate curve would have done, and it overestimated the population after a very short interval beyond the last observed ordinate as is shown in table 2.

Some 13 years ago one of the writers[5] demonstrated the applicability of a logarithmic curve of the form

$$y = a + bx + cx^2 + d \log x \qquad \text{(ii)}$$

to the representation of growth changes, using the aquatic plant *Ceratophyllum* as material. Following the application of this curve to growth of this plant it was found equally useful in representing a wide range of other growth and related changes.[6] This list now includes, of matters worked out in the Biological Laboratory of the Maine Experiment Station, such diverse phenomena as change of size of egg with successive layings, change of milk production with age, etc. Donaldson and Hatai[7] have demonstrated the applicability of this type of equation to bodily growth in the white rat and frog.

While the increase in size of a population cannot on *a priori* grounds be regarded, except by rather loose analogy, as the same thing as the growth of an organism in size, nevertheless it is essentially a growth phenomenon. It, therefore, seems entirely reasonable that this type of curve should give a more adequate representation of population increase than a simple third-order parabola. The actual event justifies this assumption, as will presently appear.

Table 1 shows the counted population as determined by the Census Bureau on the dates mentioned from 1790 to 1910. The exact dates were furnished in a personal communication from the present Director of the Census. These figures embody some adjustments and corrections made by the Census Bureau since the original censuses were made.

TABLE 1

SHOWING THE DATES OF THE TAKING OF THE CENSUS AND THE RECORDED POPULATIONS FROM 1790 TO 1910

DATE OF CENSUS		RECORDED POPULATION (REVISED FIGURES FROM STATISTICAL ABST., 1918)
Year	Month and Day	
1790	First Monday in August	3,929,214
1800	First Monday in August	5,308,483
1810	First Monday in August	7,239,881
1820	First Monday in August	9,638,453
1830	June 1	12,866,020
1840	June 1	17,069,453
1850	June 1	23,191,876
1860	June 1	31,443,321
1870	June 1	38,558,371
1880	June 1	50,155,783
1890	June 1	62,947,714
1900	June 1	75,994,575
1910	April 15	91,972,266

To the data of table 1 the following equation was fitted by the method of least squares, taking origin at 1780, and making due allowance in the abscissal intervals for the actual dates of the several censuses:

$$y = a + bx + cx^2 + d \log x$$

where y denotes population and x time. The actual equation deduced was

$$y = 9{,}064{,}900 - 6{,}281{,}430x + 842{,}377x^2 + 19{,}829{,}500 \log x. \quad \text{(iii)}$$

The results are set forth in table 2, where Pritchett's figures are given for comparison.

TABLE 2

SHOWING (*a*) THE ACTUAL POPULATION[1] ON CENSUS DATES, (*b*) ESTIMATED POPULATION FROM PRITCHETT'S THIRD-ORDER PARABOLA, (*c*) ESTIMATED POPULATION FROM LOGARITHMIC PARABOLA, AND (*d*) (*e*) ROOT-MEAN SQUARE ERRORS OF BOTH METHODS

CENSUS YEAR	(*a*) OBSERVED POPULATION	(*b*) PRITCHETT ESTIMATE	(*c*) LOGARITHMIC PARABOLA ESTIMATE	(*d*) ERROR OF (*b*)	(*e*) ERROR OF (*c*)
1790	3,929,000	4,012,000	3,693,000	+ 83,000	− 236,000
1800	5,308,000	5,267,000	5,865,000	− 41,000	+ 557,000
1810	7,240,000	7,059,000	7,293,000	− 181,000	+ 53,000
1820	9,638,000	9,571,000	9,404,000	− 67,000	− 234,000
1830	12,866,000	12,985,000	12,577,000	+ 119,000	− 289,000
1840	17,069,000	17,484,000	17,132,000	+ 415,000	+ 63,000
1850	23,192,000	23,250,000	23,129,000	+ 58,000	− 63,000
1860	31,443,000	30,465,000	30,633,000	− 978,000	− 810,000
1870	38,558,000	39,313,000	39,687,000	+ 755,000	+1,129,000
1880	50,156,000	49,975,000	50,318,000	− 181,000	+ 162,000
1890	62,948,000	62,634,000	62,547,000	− 314,000	− 401,000
1900	75,995,000	77,472,000	76,389,000	+1,477,000	+ 394,000
1910	91,972,000	94,673,000	91,647,000	+2,701,000	− 325,000
				935,000[2]	472,000[2]
1920		114,416,000	108,214,000		

[1] To the nearest thousand.

[2] Root-mean square error.

It is obvious from the data of table 2 that, with the same number of constants, the logarithmic parabola gives a distinctly better graduation than a third-order parabola.

The extreme precision of the present graduation is shown graphically in figure 1.

It is evident that as a purely empirical representation of population growth in the United States equation (iii) gives results of a very high degree of accuracy. Indeed, interpolation on this curve for inter-censal years may obviously be relied upon with a greater probability that the estimated figures approximate the unknown true facts than is afforded by any other estimating expedient hitherto applied to the known data. An indication of the general exactness of this curve (iii) for estimating future population by extrapolation may be got in the following way. Suppose a mathematician of the Civil War period had desired to estimate the population of the United States in 1910, and had fitted a curve of the type of (ii), by the method of least squares to the known data available to him, namely, the census counts of 1790, to 1860, inclusive, he would have got this result:

$$y = 8{,}619{,}800 - 5{,}680{,}540x + 822{,}709x^2 + 16{,}987{,}200 \log x \qquad \text{(iv)}$$

If he had calculated from this equation the probable population in 1910, the figure he would have obtained would have been 92,523,000, a result only approximately a half million, or 0.6%, in error, as subsequent events proved. A prophecy less than 1% in error of an event to happen 50 years later is undeniably good predicting.

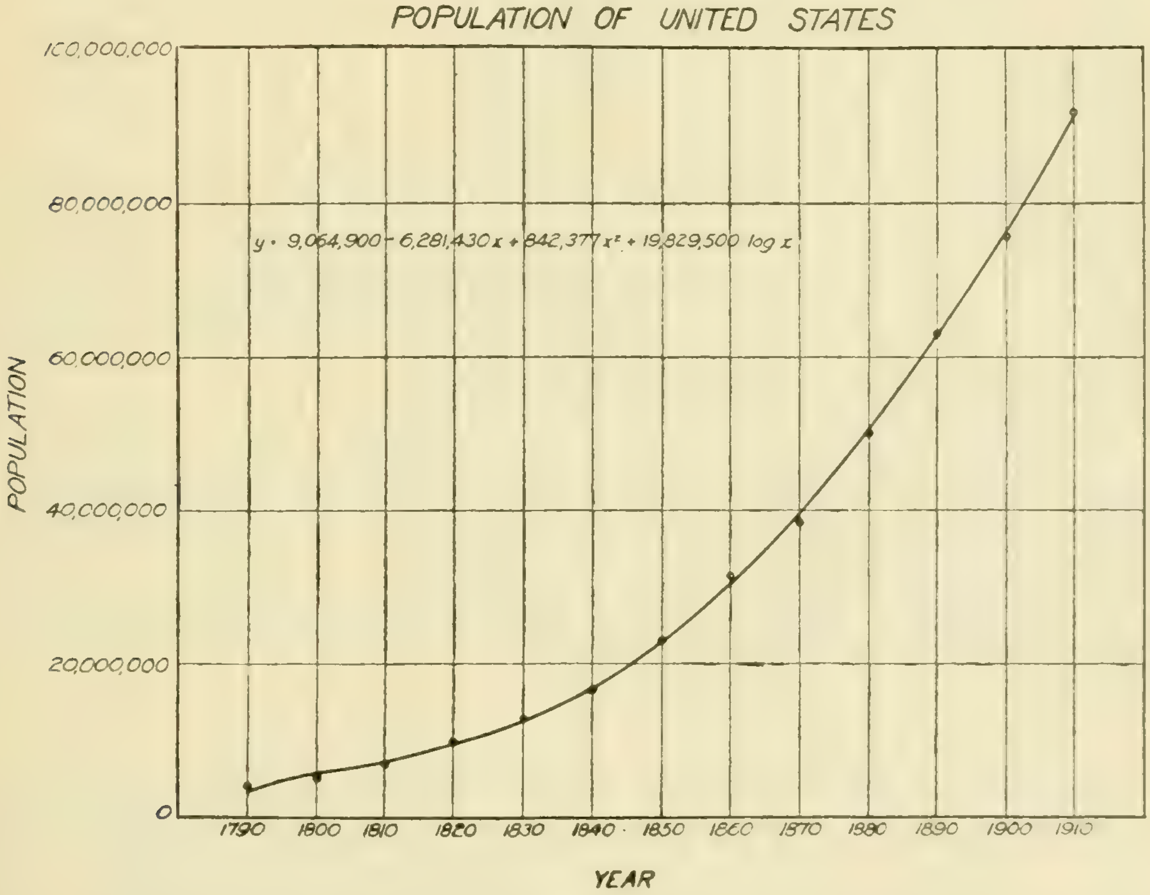

FIG. 1
Diagram showing observed and calculated populations (from logarithmic parabola) from 1790 to 1920.

It is of interest to exhibit the equations and results in predicting the 1910 population obtained by fitting our logarithmic parabola to the data available after the completion of each successive census from 1870 on. We have:

Data for 1790 to 1870, inclusive:

$$y = 8{,}287{,}700 - 5{,}300{,}270x + 795{,}540x^2 + 15{,}778{,}000 \log x \qquad \text{(v)}$$

Predicted population in 1910 = 91,201,000.
Deviation of prediction from actual, 1910 = − 771,000.
Percentage error = 0.8%.

Data for 1790 to 1880 inclusive:

$$y = 7{,}981{,}100 - 4{,}971{,}040x + 764{,}896x^2 + 14{,}993{,}500 \log x \qquad \text{(vi)}$$

Predicted population in 1910 = 89,128,000.
Deviation of prediction from actual, 1910 = – 2,844,000.
Percentage error = 3%.

Data for 1790 to 1890, inclusive:

$$y = 9,013,800-6,242,170x + 839,782x^2 + 19,744,300 \log x \qquad \text{(vii)}$$

Predicted population in 1910 = 91,573,000.
Deviation of prediction from actual, 1910 = – 399,000.
Percentage error = 0.4 per cent.

Data for 1790 to 1900 inclusive:

$$y = 8,748,000-5,880,890x + 821,001x^2 + 18,232,100 \log x \qquad \text{(viii)}$$

Predicted population in 1910 = 91,148,000.
Deviation of prediction from actual, 1910 = –824,000.
Percentage error = 0.9%.

Beginning with 1860 (equation (iv)) and coming down to 1900, our hypothetical statistician would have been only once in error as much as 1% in his prediction of the 1910 population by this logarithmic parabola. The one larger error is for the 1880 curve, where apparently the aberrant counts of 1860 and 1870 exert an undue influence.

Altogether it seems justifiable to conclude that:

1. A logarithmic parabola of the type of equation (ii) describes the changes which have occurred in the population of the United States in respect of its gross magnitude, with a higher degree of accuracy than any empirical formula hitherto applied to the purpose.

2. The accuracy of the graduation given by this logarithmic parabola is entirely sufficient for all practical statistical purposes.

II

Satisfactory as the empirical equation above considered is from a practical point of view, it remains the fact that it is an empirical expression solely, and states no general law of population growth. Insofar it is obviously an undesirable point at which to leave the problem of the mathematical expression of the change of population in magnitude.

It is quite clear on *a priori* grounds, as was first pointed out by Malthus in non-mathematical terms, that in any restricted area, such as the United States, a time must eventually come when population will press so closely upon subsistence that its rate of increase per unit of time must be reduced to the vanishing point. In other words, a population curve may start, as does that shown in figure 1, with a convex face to the base, but presently it must develop a point of inflection, and from that point on present a concave face to the x axis, and finally become asymptotic, the asymptote representing the maximum number of people which can be supported on the given fixed area.[8] Now, while an equation like (ii) can, and will in due time, develop a point of inflection and become concave to the base it never can become asymptotic. It, therefore, cannot be regarded as a hopeful line of approach to a true law of population growth.

What we want obviously is a mathematical picture of the *whole* course of population in this country. It is not enough to be able to predict twenty or fifty years ahead as our logarithmic parabola is able to do satisfactorily, *in one portion of the whole curve.* How absurd equation (iii) would be over a really long time range is shown if we attempt to calculate from it the probable population in, say, 3000 A.D. It gives a value of 11,822,000,000. But this is manifestly ridiculous; it would mean a population density of 6.2 persons per acre or 3968 persons per square mile.

It would be the height of presumption to attempt to predict *accurately* the population a thousand years hence. But any real law of population growth ought to give some general and approximate indication of the number of people who would be living at that time within the present area of the United States, provided no cataclysmic alteration of circumstances has in the meantime intervened.

It has seemed worth while to attempt to develop such a law, first by formulating a hypothesis which rigorously meets the logical requirements, and then by seeing whether in fact the hypothesis fits the known facts. The general biological hypothesis which we shall here test embodies as an essential feature the idea that the rate of population increase in a limited area at any instant of time is proportional (*a*) to the magnitude of the population existing at that instant (amount of increase already attained) and (*b*) to the still unutilized potentialities of population support existing in the limited area.

The following conditions should be fulfilled by any equation which is to describe adequately the growth of population in an area of fixed limits.

1. Asymptotic to a line $y = k$ when $x = +\infty$.
2. Asymptotic to a line $y = 0$ when $x = -\infty$.
3. A point of inflection at some point $x = \alpha$ and $y = \beta$.
4. Concave upwards to left of $x = \alpha$ and concave downward to right of $x = \alpha$.
5. No horizontal slope except at $x = \pm\infty$.
6. Values of y varying continuously from 0 to k as x varies from $-\infty$ to $+\infty$.

In these expressions y denotes population, and x denotes time.

An equation which fulfils these requirements is

$$y = \frac{be^{ax}}{1 + ce^{ax}} \qquad \text{(ix)}$$

when a, b and c have positive values.

In this equation the following relations hold:

$$x = +\infty \qquad y = \frac{b}{c} \qquad \text{(x)}$$

$$x = -\infty \qquad y = 0 \qquad \text{(xi)}$$

Relations (x) and (xi) define the asymptotes.

The point of inflection is given by $1-ce^{ax} = 0$, or

$$x = -\frac{1}{a}\log c \qquad y = \frac{b}{2c} \qquad \text{(xii)}$$

The slope at the point of inflection is $\frac{ab}{4c}$.

Expressing the first derivative of (xi) in terms of y, we have

$$\frac{dy}{dx} = \frac{ay(b-cy)}{b}. \qquad \text{(xiii)}$$

Putting the equation in this form shows at once that it is identical with that describing an autocatalyzed chemical reaction, a point to which we shall return later.

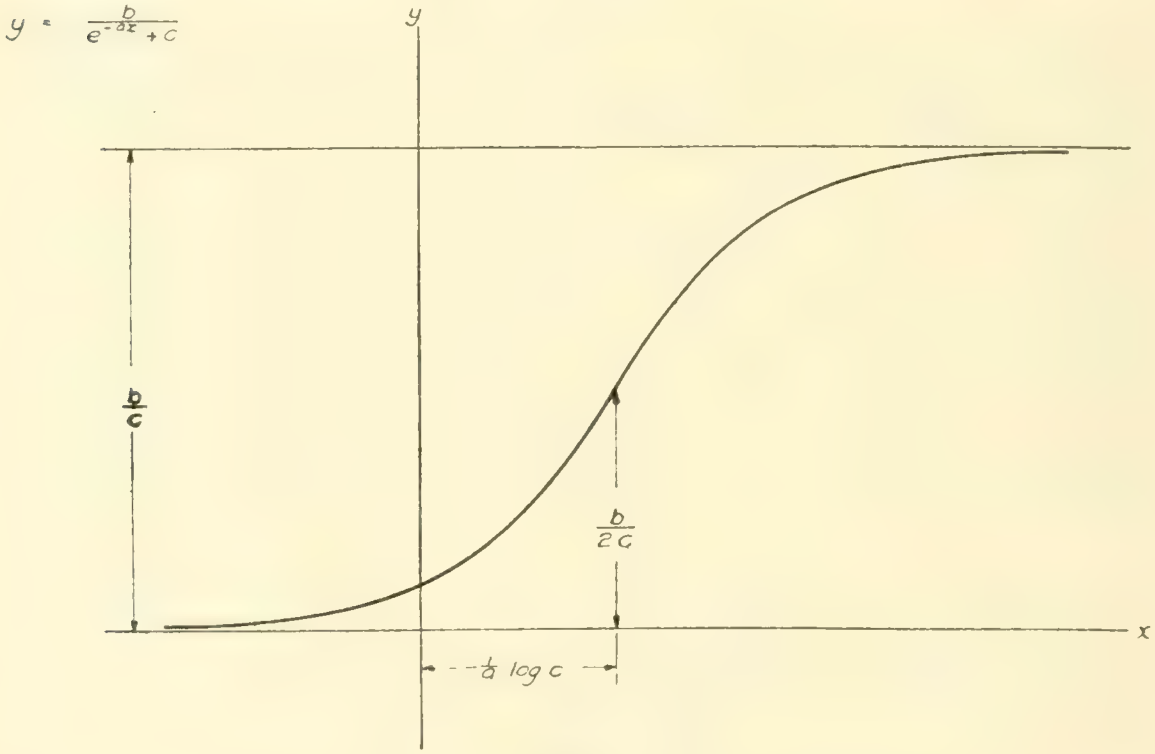

FIG. 2

General form of curve given by equation (ix).

The general form of the curve is shown in figure 2.

The question now is how well does (ix) represent the known historical facts as to the growth in population of the United States, and to what legitimate deductions as to the future course of population in this country does it lead?

It is obvious that equation (ix) as it stands cannot be fitted to observational data by the method of least squares. It is possible to write momental equations and fit by the method of moments, but at this tim we do not care to develop that method because, as will presently appear we do not regard equation (ix) as the final development of this type of equation for representing population, and we have no desire to encumber the literature with a mathematical discussion which we expect later to discard.

For present purposes it will be sufficient to fit (ix) to the observations by passing it through three points. Given three equally spaced ordinates, y_1, y_2 and y_3, the necessary equations are:

$$\frac{b}{c} = \frac{2y_1y_2y_3 - y_2^2(y_1 + y_3)}{y_1y_3 - y_2^2} \tag{xiv}$$

$$a = \log_{10} \frac{y_2\left(\frac{b}{c} - y_1\right)}{y_1\left(\frac{b}{c} - y_2\right)} \div h \log_{10} e \tag{xv}$$

where h is the abscissal distance in years between y_1 and y_2, or y_2 and y_3.

$$c = \frac{1}{y_2 - y}\left(\frac{y_1}{e^{a\alpha}} - \frac{y_2}{e^{a(\alpha+h)}}\right) \tag{xvi}$$

where α is the abscissal distance in years from the origin to y_1.

Putting x_1 at 1790, x_2 at 1850, and x_3 at 1910, and taking origin at 1780 we have

$y_1 = 3{,}929$[9]

$\alpha = 10$

$y_2 = 23{,}192$

$h = 60$

$y_3 = 91{,}972$

and taking (ix) in the form

$$y = \frac{b}{e^{-ax} + c}, \tag{xvii}$$

we find these values for the constants:

$$y = \frac{2{,}930.3009}{e^{-.0313395x} + 0.014854} \tag{xviii}$$

The closeness with which this curve fits the known facts is shown in table 3.

The closeness of fit of this curve is shown graphically in figure 3.

Though empirically arrived at this is a fairly good fit of theory to observations. The root-mean square error from the last column is 463,000, or slightly smaller than that from the logarithmic parabola in table 2. It must not be forgotten, however, that the root-mean square error is reduced in the present case by virtue of the fact that in three out of the 13 ordinates theory and observation are made, by the procrustean method of fitting, to coincide exactly. The most that can be asserted is that

TABLE 3

RESULTS OF FITTING POPULATION DATA 1790 TO 1910 BY EQUATION (XVIII)

YEAR	OBSERVED POPULATION	CALCULATED POPULATION BY EQUATION (XVIII)	ERROR
1790	3,929,000	3,929,000	0
1800	5,308,000	5,336,000	+ 28,000
1810	7,240,000	7,228,000	— 12,000
1820	9,638,000	9,757,000	+119,000
1830	12,866,000	13,109,000	+243,000
1840	17,069,000	17,506,000	+437,000
1850	23,192,000	23,192,000	0
1860	31,443,000	30,412,000	—1,031,000
1870	38,558,000	39,372,000	+ 814,000
1880	50,156,000	50,177,000	+ 21,000
1890	62,948,000	62,769,000	— 179,000
1900	75,995,000	76,870,000	+ 875,000
1910	91,972,000	91,972,000	0

equation (xviii) gives nearly or quite as good a fit to the observations as does the logarithmic parabola. If we properly graduated the data, by the method of moments, we should probably get a result measurably better than that from equation (iii).

The significance of the result lies in this consideration. A curve which on *a priori* grounds meets the conditions which must be satisfied by a true law of population growth, actually describes with a substantial degree of accuracy what is now known of the population history of this country.

Let us examine some further consequences which flow from equation (xviii). The first question which interests one is this: when did or will the population curve of this country pass the point of inflection, and exhibit a progressively diminishing instead of increasing rate of growth? From (xii) it is easily determined that this point occurred about *April 1, 1914*, on the assumption that the numerical values of (xviii) reliably represent the law of population growth in this country. In other words, so far as we may rely upon present numerical values, the United States has already passed its period of most rapid population growth, unless there comes into play some factor not now known and which has never operated during the past history of the country to make the rate of growth more rapid. This latter contingency is improbable. While prophecy is a dangerous pastime, we believe, from the fragmentary results already announced, that the 1920 census will confirm the result indicated by our curve, that the period of most rapid population growth was passed somewhere in the last decade. The population at the point of inflection works out to have been 98,637,000, which was in fact about the population of the country in 1914.

The upper asymptote given by (xviii) has the value 197,274,000 roughly. This means that according to equation (xviii) the maximum population which continental United States, as now areally limited, will ever have will be roughly twice the present population. We fear that some will condemn at once the whole theory because the magnitude of this number is not sufficiently imposing. It is so easy, and most writers on population have been so prone, to extrapolate population by geometric series, or by a parabola or some such purely empirical curve, and arrive at stupendous figures, that calm consideration of real probabilities is most difficult to obtain. While, as will appear from the next section of this paper, we have no desire to defend the numerical results of this section, and indeed ourselves regard them only as a rough first approximation, it remains a fact that if anyone will soberly think of every city, every village, every town in this country having its present population multiplied by 2, and will further think of twice as many persons on the land in agricultural pursuits, he will be bound, we think, to conclude that the country would be fairly densely populated. It would have about 66 persons per square mile of land area.

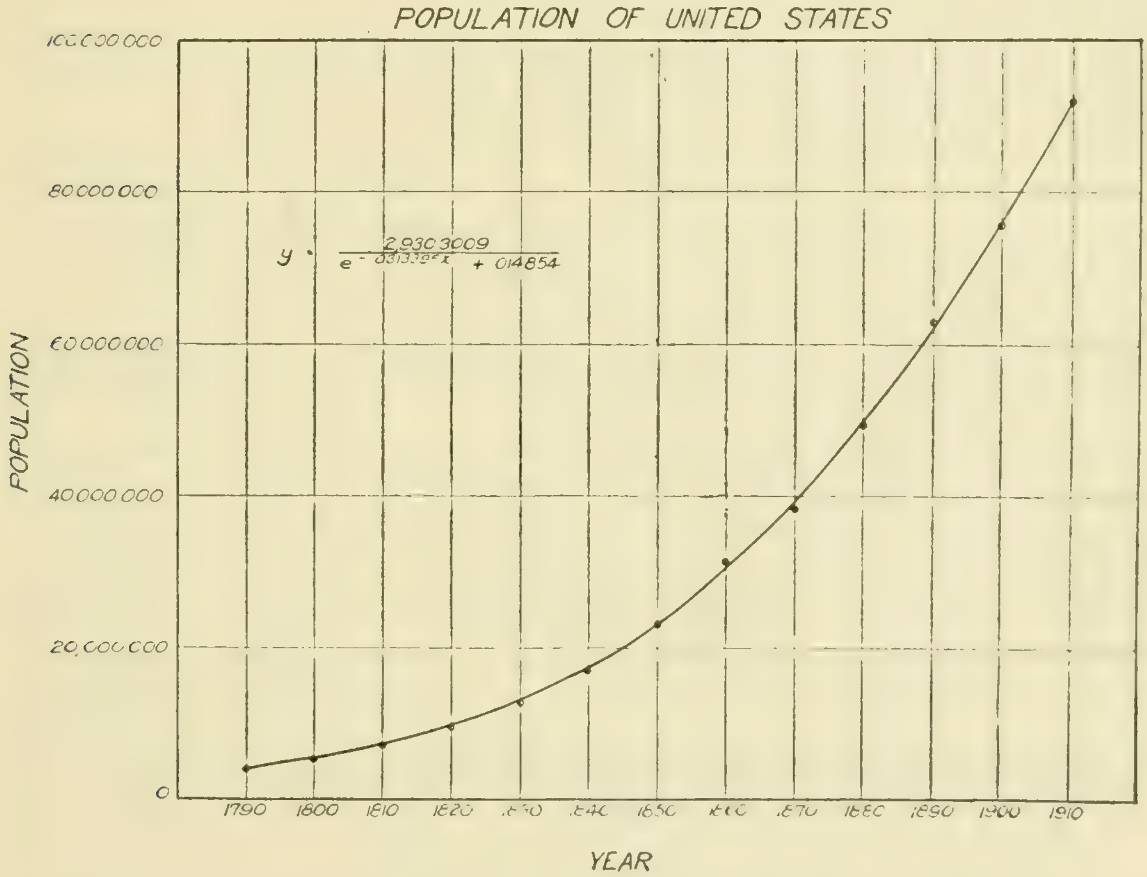

FIG. 3

Showing result of fitting equation (xviii) to population data.

It will at once be pointed out that many European countries have a much greater density of population than 66 persons to the square mile.

as for example Belgium with 673, Netherlands with 499, etc. But it must not be forgotten that these countries are far from self-supporting in respect of physical means of subsistence. They are economically self-supporting, which is a very different thing, because by their industrial development at home and in their colonies they produce money enough to buy physical means of subsistence from less densely populated portions of the world. We can, of course, do the same thing, provided that by the time our population gets so dense as to make it necessary there still remain portions of the globe where food, clothing material, and fuel are produced in excess of the needs of their home population. But in this, and in any other scientific discussion of population, it is necessary to limit sharply the *area* one is to talk about. This paper deals with population, and by direct implication the production of physical means of subsistence, within the present area of continental United States.

Now 197,000,000 people will require, on the basis of our present food habits,[10] about 260,000,000,000,000 calories per annum. The United States, during the seven years 1911–1918, produced as an annual average, in the form of human food, *both primary and secondary* (i.e., broadly vegetable and animal), only 137,163,606,000,000 calories per year.[11] So that unless our food habits radically change, and a man is able to do with less than 3000 to 3500 calories per day, or unless our agricultural production radically increases,[12] it will be necessary when our modest figure for the asymptotic population is reached, to import nearly or quite one-half of the calories necessary for that population. It seems improbable that the population will go on increasing at any very rapid rate after such a condition is reached. And is it at all reasonable to suppose that at such time, with all the competition for means of subsistence which the already densely populated countries of Europe will be putting up, there can be found any portion of the globe producing food in excess of its own needs to an extent to make it possible for us to find the calories we shall need to import?

Altogether, we believe it will be the part of wisdom for anyone disposed to criticise out asymptotic value of a hundred and ninety-seven and a quarter millions because it is thought too small, to look further into all the relevant facts.

III

With the above numerical results in hand it is desirable to discuss a little further the general theory of population growth set forth in the preceding section. At the outstart let it be said that we are convinced that equation (ix) represents no more than a first approximation to a true law of population growth. There are several characteristics of this curve which are too rigid and inelastic to meet the requirements of such a law. In (ix) the point of inflection must of necessity lie exactly half-way between the two asymptotes. Furthermore the half of the curve lying to the right of the point of inflection is an exact reversal of the half lying to the left of that point. This implies that the forces which during the latter part of the population history of an area act to inhibit the rate of population growth are equal in magnitude, and exactly similarly distributed in time, to the forces which in the first half of the history operate to accelerate growth. We do not believe that such rigid and inelastic postulates as these are, in fact, realized in population growth.

The same objections apply to the use of the equation of an autocatalytic reaction to the representation of organic growth in the individual. This fact has been noted by Robertson[13] who was the first to discover that, in general, growth follows much the same curve as that of autocatalysis. What needs to be done is to generalize (ix) in some such form as will free it from the two restrictive features (location of point of inflection and symmetry) we have mentioned, and will at the same time retain its other essential features. We are working along this line now and hope presently to reach a satisfactory solution.

We attach no particular significance to the *numerical* results of the preceding section. They obviously can give only the roughest approximation to probable future values of the population of the United States. Our only purpose in presenting them at all at this time is to demonstrate that the hypothesis here advanced as to the law of population growth, even when fitted by a rough and inadequate method, so closely describes the known facts regarding the past history of that growth, as to make it potentially profitable to continue the mathematical development and refinement of this hypothesis further. There is much that appeals to the reason in the hypothesis that growth of population is fundamentally a phenomenon like autocatalysis. In a new and thinly populated country the population already existing there, being impressed with the apparently boundless opportunities, tends to reproduce freely, to urge friends to come from older countries, and by the example of their well-being, actual or potential, to induce strangers to immigrate. As the population becomes more dense and passes into a phase where the still unutilized potentialties of subsistence, measured in terms of population, are measurably smaller than those which have already been utilized, all of these forces tending to the increase of population will become reduced.

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[1] Papers from the Department of Biometry and Bital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 13.

[2] Cf. for a discussion on the relation of curve fitting to true organic laws of change. Pearl, R. "Some Recent Studies on Growth," *Amer. Nat.*, **43,** 1909 (302–316).

[3] Perrin, E., "On Some Dangers of Extrapolation," *Biometrika*, **3,** 1904 (99 103).

[4] Pritchett, A. S., "A Formula for Predicting the Population of the United States," *Quart. Publ. Amer. Statistical Assoc.*, **2,** 1891 (278–286).

[5] Pearl, R., "Variation and Differentiation in Ceratophyllum," *Carnegie Inst. Washington, Publ.* **58,** 1907 (136).

[6] Cf. the following papers:

Curtis, M. R., 1914, "A Biometrical Study of Egg Production in the Domestic Fowl. IV. Factors Influencing the Size, Shape, and Physical Constitution of Eggs," *Arch. Entwicklungsmech. Organ.*, B. **39**, Heft 2/3, pp. 217–327.

Pearl, R., 1909, "Studies on the Physiology of Reproduction in the Domestic Fowl. I. Regulation in the Morphogenetic Activity of the Oviduct," *J. Exp. Zool.*, **6**, No. 3, pp. 339–359.

Pearl, R., 1914, "On the Law Relating Milk Flow to Age in Dairy Cattle," *Proc. Soc. Expt. Biol. Med.*, **12**, No. 1, pp. 18–19.

[7] Donaldson, H. H., 1908, "A Comparison of the Albino Rat with Man in Respect to the Growth of the Brain and of the Spinal Cord," *J. Compar. Neurol. Psych.*, **18**, No. 4, pp. 345–389.

Donaldson, H. H., 1909, "On the Relation of the Body Length to the Body Weight and to the Weight of the Brain and of the Spinal Cord in the Albino Rat (*Mus norvegicus var. albus*)," *Ibid.*, **19**, No. 2, pp. 155–167.

Donaldson, H. H., 1910, "On the Percentage of Water in the Brain and in the Spinal Cord of the Albino Rat," *Ibid.*, **20**, No. 3, pp. 119–144.

Donaldson, H. H., 1911, "On the Regular Seasonal Changes in the Relative Weight of the Central Nervous System of the Leopard Frog," *J. Morph.*, **22**, pp. 663–694.

Donaldson and Hatai, Shinkishi, 1911, "A Comparison of the Norway Rat with the Albino Rat in Respect to Body Length, Brain Weight, Spinal Cord Weight, and the Percentage of Water in Both the Brain and the Spinal Cord," *J. Compar. Neurol. Psych.*, **21**, pp. 417–458.

Hatai, Shinkishi, 1909, "Note on the Formulas Used for Calculating the Weight of the Brain in the Albino Rats," *Ibid.*, **19**, No. 2, pp. 169–173.

Hatai, Shinkishi, 1911, "A Formula for Determining the Total Length of the Leopard Frog (*R. pipiens*) for a Given Body Weight," *Anat. Rec.*, **5**, No. 6, pp. 309–312.

Hatai, Shinkishi, 1911, "An Interpretation of Growth Curves from a Dynamical Standpoint," *Ibid.*, **5**, No. 8, pp. 373–382.

[8] Always, be it clearly understood, on the assumption that the average standard of living, method of agricultural production, etc., either do not further change at all in the period between the end of historical past record of fact and the time when dy/dx becomes negligibly small as the asymptote is approached, or that the net effective magnitude of any such changes as do occur will be relatively so small as to be negligible in comparison with the effect of such factors as reproduction and immigration in determining the relation between population and time in an area of fixed limits. In any mathematical treatment of the subject these factors of standard of living, methods of agriculture, etc., represent essentially constant (and hence omitted) parameters of any assumed functional relation between population and time.

[9] Omitting 000 here and in the subsequent calculations till the end.

[10] Cf. Pearl, R., *The Nation's Food*, Philadelphia (W. B. Saunders Company), 1920 (247).

[11] Pearl, R., *loc. cit.*, p. 76.

[12] As a matter of fact East, in his able presidential address on "Population," before the American Society of Naturalists has shown that the United States has already entered upon the era of diminishing returns in agriculture in this country.

[13] Robertson, T. Brailsford, "On the Normal Rate of Growth of an Individual and Its Biochemical Significance," *Arch. Entwickmech. Organ.*, **25**, pp. 581–614.

Robertson, T. Brailsford, "Further Remarks on the Normal Rate of Growth of an Individual, and Its Biochemical Significance," *Ibid.*, **26**, pp. 108–118.

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ON A SINGLE NUMERICAL INDEX OF THE AGE DISTRIBUTION OF A POPULATION[1]

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Read before the Academy, April 27, 1920

It is an obvious fact that the crude death rate of any community is influenced in a marked degree by the age distribution of the living population of that community. Before any critical conclusions about the true force of mortality can be drawn some sort of correction must be applied to take account of the age distribution of the population. If one desires to make any analysis of the correlation of death rates with each other or with sundry environmental factors it is an absolutely essential prerequisite that there be found some single numerical expression which will be an approximate index of the age distribution of the population of each locality dealt with.

From a mathematical viewpoint the problem presented is one incapable of *exact* solution. The problem mathematically is to find a single parameter which will fully describe so complex a curve as that of the age distribution of a population, the general form of which is indicated in figure 1. But this is impossible. No single parameter can possibly describe fully such a curve. The best that can be done is to approximate as closely as may be to the impossible ideal. It is the purpose of this paper to describe and illustrate an approximation which comes very close to the requirements, indeed quite sufficiently so for all practical statistical purposes in all cases where it has yet been tested out, and is easy to calculate.

In a recent paper,[2] I proposed as an index of differences in age composition of populations, and applied it to 40 American cities, the expression

$$\chi^2 = S\left(\frac{\Delta^2}{P}\right) \qquad (1)$$

"where Δ is the deviation for each of six age groups (viz, 0-4, 5-14, 15-24, 25-44, 45-64, 65 and over) of the percentage of the actual population of each city in 1910 in each age group, from the percentage in the same group in the Standard Population of Glover's[3] Life Table, denoted in the formula by P. S denotes summation of all six values. The value χ^2 measures the extent to which each city deviates in the age constitution of its population from a fixed standard, but does not tell the nature or kind of the deviation."

In the paper cited it was shown empirically that in spite of the obvious defect of this index mentioned in the last sentence, it was adequate for the restricted material and purposes there involved. Since that paper was published it has occurred to me that the outstanding defect of the former index will, to that degree of accuracy which is requisite for all practical statistical purposes, be entirely removed by adopting as an age-constitution index the function

$$\phi = S\left\{\frac{\Delta^2}{P}\right\}(M-M_p) \qquad (2)$$

where S, Δ and P have the same significance as before, and M = mean age of living population in any community, M_p = mean age of persons in a stationary population unaffected by migration and which, assuming the mortality rates of Glover's Life Table, would result if 100,000 persons were born alive uniformly throughout each year (M_p calculated from L_x line of Glover's Table (p. 16) = 33.796 years).

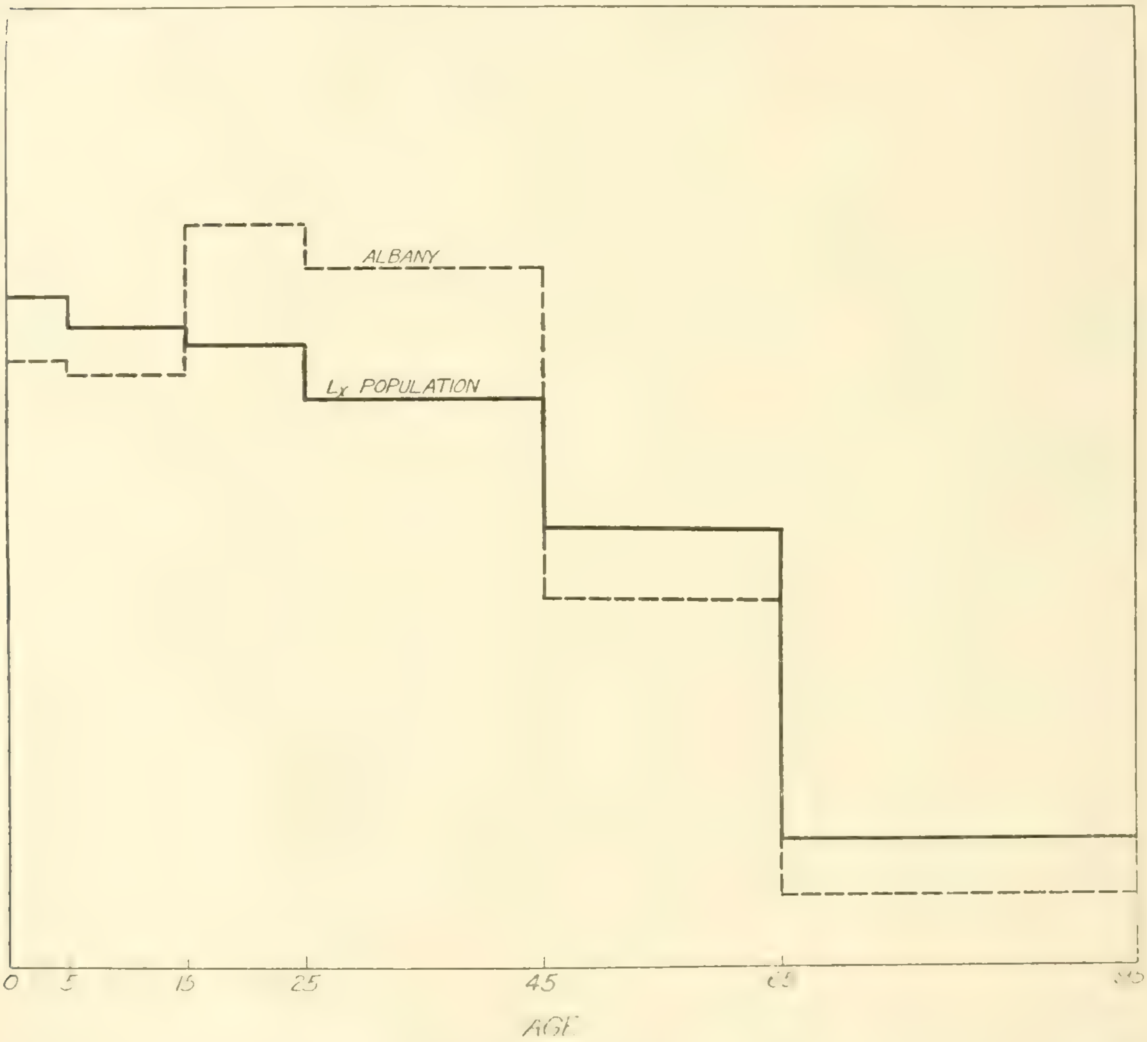

FIG. 1. Diagram showing age distribution of population, grouped in six-age classes. The last class in this and other diagrams is assumed to have its ending at age 95. The solid line gives the stationary life table population, and the broken line the population of Albany. Total area under each curve = 100%.

This procedure simply multiplies our former index χ^2 by the difference (given its proper sign) between the mean age of the observed population and the mean age of the standard population on the basis of which χ^2 was calculated. Since in fact the mean age of any actual urban population is never likely to be as great as the mean age of the stationary population chosen as a standard of reference the actual values of ϕ will practically always be negative for cities. The smaller these negative values are numerically the greater will be the proportion of older persons in the population concerned. In short this function ϕ tells us not only the degree to which a given population deviates in its age distribution from a fixed standard age distribution, but also the nature of this deviation, whether on the one hand in the direction of a relative excess of aged, or on the other hand in the direction of a relative excess of the young. Theoretically it is possible for two populations differing from one another in a compensatory way to give the same values for the index ϕ. But two populations which differ in age distribution in any fundamental respect which could affect appreciably crude death rates will, in all populations I have been able to test, give different values of ϕ, provided the age classification from which the function is calculated is finely enough divided.

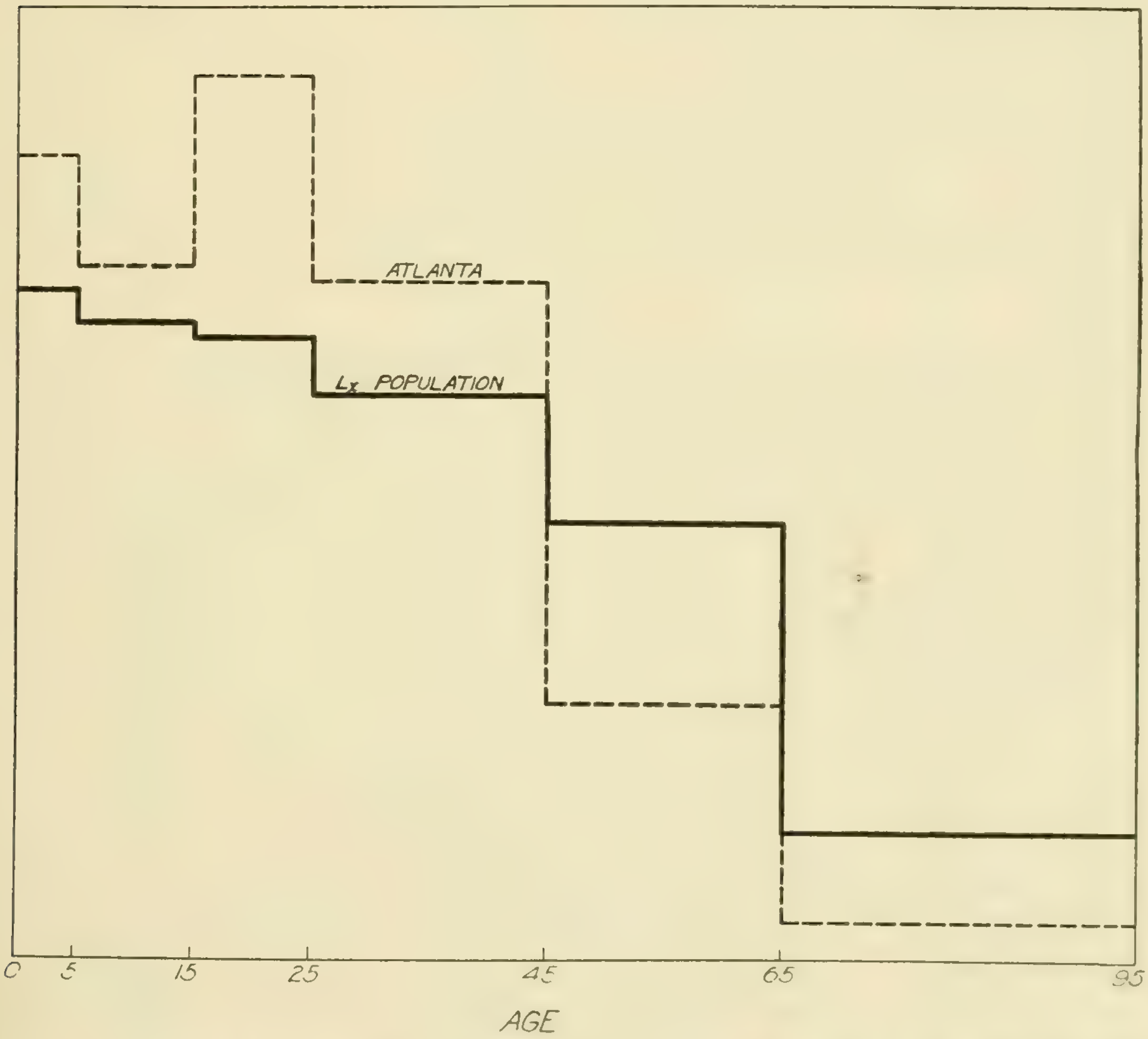

FIG. 2. Diagram showing age distribution of population, grouped in six age classes. The solid line gives the stationary life-table population, and the broken line the population of Atlanta. Total area under each curve = 100%.

II

The numerical values of the index ϕ for a series of American cities, on the basis of the age distribution of the 1910 census are given by way of illustration in table 1. The cities are arranged in descending order of departure from the L_x population.

The extent of the departures from the standard population denoted by the several values of the indices may be indicated graphically by plotting the age distribution curve, on a percentage basis, for the two extreme cities in the table, Albany and Atlanta, against the L_x population. This is done in figures 1 and 2.

The general characteristics of an urban population as compared with a stationary life table population are well shown in these diagrams. In general a city has population under 40 to 45 in excess, and population older than that in defect. The reason is obvious.

TABLE 1

SHOWING AGE-CONSTITUTION INDICES OF THE POPULATION OF AMERICAN CITIES

CITY	ϕ	CITY	ϕ
Albany	−10.73	Lowell	−34.91
Los Angeles	−13.61	Philadelphia	−39.51
Oakland	−17.18	San Francisco	−43.35
Washington	−20.67	St. Louis	−44.57
Cincinnati	−22.93	Nashville	−48.32
Dayton	−24.10	Buffalo	−48.61
Indianapolis	−24.23	New Orleans	−52.44
Rochester	−26.62	Minneapolis	−55.39
Grand Rapids	−27.95	Milwaukee	−62.17
Columbus	−29.08	Newark	−62.54
Providence	−30.05	Chicago	−68.76
Cambridge	−30.26	St. Paul	−68.95
Boston	−31.05	Pittsburgh	−71.77
Baltimore	−31.80	Fall River	−73.39
Louisville	−32.19	New York	−74.42
Toledo	−33.21	Cleveland	−74.51
New Haven	−33.70	Atlanta	−82.71

III

It will be well to see in detail how the age distributions of some cities having nearly the same value for ϕ compare in respect of actual age distribution of their populations. Table 2 furnishes such comparisons for four pairs of cities, covering fairly the whole rank of values of ϕ shown in table 1.

From this table it is seen that as we pass from cities having a value of ϕ of about 24 to cities having ϕ equal to about 74 there is a steady change from populations having relatively many persons in the higher age groups

TABLE 2

PERCENTAGE AGE DISTRIBUTION OF THE POPULATION OF CERTAIN CITIES IN 1910

AGE CLASSES (YEARS)	DAYTON	INDIANAPOLIS	PROVIDENCE	CAMBRIDGE	NASHVILLE	BUFFALO	NEW YORK	CLEVELAND
	$\phi = -24.10$	$\phi = -24.23$	$\phi = -30.05$	$\phi = -30.26$	$\phi = -48.32$	$\phi = -48.61$	$\phi = -74.42$	$\phi = -74.51$
0– 4	9.1%	8.0%	9.7%	10.3%	9.2%	10.0%	10.6%	11.1%
5–14	15.4	15.3	16.5	17.5	17.8	18.3	18.1	17.4
15–24	19.5	19.4	19.0	18.4	21.9	20.6	20.8	20.5
25–44	34.6	35.9	34.2	33.3	32.2	32.3	33.9	34.4
45–64	17.0	17.0	16.2	16.0	15.1	15.5	13.7	13.4
65 & over	4.4	4.3	4.2	4.4	3.8	3.4	2.8	3.0
Totals	100.0	99.9	99.8	99.9	100.0	100.1	99.9	99.8

to populations having relatively few persons in those groups. Furthermore, it is evident that in each of the four pairs of cities compared the agreement between the two cities having nearly identical values of ϕ is very close in respect of actual percentage distribution of the population. Dayton and Indianapolis were for all practical statistical purposes identical in the age distribution of their populations in 1910. So were Providence and Cambridge, Nashville and Buffalo, and New York and Cleveland. In each case the curve for one distribution of the pair winds in and out about the path set by the other. Of course, we should get finer differentiations brought out by the ϕ function if we used 15 or 20 age classes instead of the 6 here employed. But for the purposes of the investigation in which I am using these functions, and for purposes of illustration of method 6 classes are sufficient. A word of caution must, however, be emphasized here. The reliability of ϕ as an approximate index of differences in age distributions of population is greater as we pass in either direction towards the ends of its range of values. In the case of populations giving values of ϕ near the mean (say in the thirties for American cities) it may be necessary in order to get really differentiant values to calculate ϕ from a rather fine age grouping.

In general it is believed that the function of an age distribution of a population here proposed will give, in a single numerical expression, a substantially accurate indication of the essential nature of that age distribution, and will facilitate the differentiation and classification of populations in respect of this characteristic for statistical studies, particularly by the method of multiple correlation.

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[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 16.

[2] Pearl, R., "Influenza Studies. I—On Certain General Statistical Aspects of the 1918 Epidemic in American Cities," *Public Health Repts.*, **34,** 1919 (1743–1783).

[3] Glover, J. W., *United States Life Tables*, 1910, Bureau of the Census, 1916.

VARIATION IN THE RATE OF INFANT MORTALITY IN THE UNITED STATES BIRTH REGISTRATION AREA [1] †

RAYMOND PEARL, Ph.D., Baltimore

Until recently it has been impossible to discuss on any accurate or satisfactory basis the infant mortality of any considerable portion of the United States. This difficulty has arisen from the fact that except in a few localities, notably some of the New England States, there has been in the past no adequate system of birth registration. The most accurate practical method of stating the force of infant mortality is to relate the number of deaths of infants under one year of age in a given time unit to the number born in the same time unit. Consequently, one needs accurate birth statistics before infant mortality can be adequately discussed.

It is a matter of great satisfaction to everyone interested in the subject of infant mortality that at last there is well established a Birth Registration Area for the United States, and four annual reports on Birth Statistics of this area have been issued to date by the Census Bureau. We are well embarked now on the policy of adequate birth statistics for the country and unquestionably within a comparatively few years the Birth Registration Area will cover the major portion of the country as the Death Registration Area now does. In the short period since the Birth Registration Area has been established its growth in extent has been gratifyingly rapid. The first report on birth statistics for the year 1915 comprised data from an area including approximately 31 per cent of the population of the country. The 1918 birth statistics report gives data from an area including 53 per cent of the population. This furnishes a sufficient volume of material so that one may begin the mathematical analysis of some of the problems of infant mortality with some assurance of reaching valid conclusions.

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[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 18. This paper, which is a preliminary and condensed abstract of a much more detailed investigation of the subject, shortly to be published elsewhere.

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† Reprinted from the Transactions of the Eleventh Annual Meeting of the American Child Hygiene Association, St. Louis, October 11-13, 1920.

The purpose of the present paper is a modest one. It aims simply to present briefly some of the facts of variation in rate of infant mortality in different geographic or demographic units of the population. The first step in the solution of any problem is obviously a clear definition of the problem itself. We shall see, as we pass from city to city, town to town, or rural county to rural county, that the rate of infant mortality varies greatly. In a hypothetical commonwealth where the most perfect administrative control over infant mortality possible or conceivable had been attained, this variation would largely disappear, the only residue of diversity between communities in respect of infant mortality being such as arose purely by the operation of chance, that is, from random sampling, or from uncontrollable environmental factors, such as climate or soil. Now, with the actually existing condition of variation between different communities in respect of infant mortality, it is obvious that there must be particulate and presumably determinable reasons for each particular difference which exists. Operating on a basis largely of empiricism and *a priori* reasoning, efforts to reduce infant mortality have in the past been attended with considerable success. Also, with the advance of general sanitation the death rate under one year of age has fallen enormously. Greenwood [2] quotes some interesting figures on the point from Farr, which we may well reproduce here to show how enormous has been the improvement.

Period	1730-49	1750-69	1770-89	1790-1809	1810-29
Percentage Deaths under 5 years....	74.5	63.0	51.5	41.3	31.8

But after such a decline as these figures indicate the continuation of the business offers a difficult problem to the administrative official, whose procedures are grounded essentially only on the two pedestals of what he thinks *has* worked in the past and what he believes logically *ought* to work. The easy part of the conflict has happened and is in the past. To continue the good fight with the same relative measure of success, one presently must needs know more precisely than is now known the pattern of the causal nexus which controls and determines the rate of infant mortality. And it is *real* knowledge, not *a priori* logic, that is wanted. Let a single example illustrate. It has been maintained that excessive infant mortality is primarily the resultant of the so-called "degrading influences" of poverty, and such a contention stirs a warmly sentimental feeling of agreement in the minds of the well-meaning public zealous to do good. This relationship obviously *ought* to be true, therefore to a too-common type of mind it must be and is true. But Greenwood and Brown[8] in what may fairly be regarded the most thoroughly sound, critical and penetrating contribution which has yet been made to the problem of infant mortality are unable "to demonstrate any unambiguous association between poverty . . . and the death rate of infants."

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[2] Greenwood, M. Infant mortality and its administrative control. Eugenics Rev. Oct. 1912, pp. (of reprint) 1-23.

The plain fact is that before control or ameliorative measures can be applied with the maximum of efficient economy to the general public health problem of infant mortality we must know a great deal more than we now do about the factors which induce spatial and temporal differences in the rate of that mortality. But first we must get an adequate conception of the magnitude and character of the differences themselves. Let us, therefore, turn to the examination of the facts regarding variation in infant mortality in the United States Birth Registration Area.

Variation Data

In this work we have studied the variation in the rate of infant mortality (deaths per thousand births) for the following groups:

1. Total population in cities of population of 25,000 or over in 1910.
2. Total population in cities of under 25,000 population in 1910.
3. Total population in rural counties of registration states.
4. White population in cities of population of 25,000 or over in 1910.
5. White population in cities of under 25,000 population.
6. White population in rural counties of registration states.
7. Colored population in cities of population of 25,000 or over in 1910.
8. Colored population in cities of under 25,000 population.
9. Colored population in rural counties of registration states.

In order to make possible a better appreciation of the nature of the frequency distributions Figure 1 has been prepared. This shows for the year 1918 the frequency polygons for the total population of (a) cities of 25,000 and over, (b) cities of under 25,000, and (c) rural counties.

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[8] Greenwood, M. and Brown, J. W. An examination of some factors influencing the rate of infant mortality. Jour. Hyg. Vol. XII. pp. 5-45, 1912.

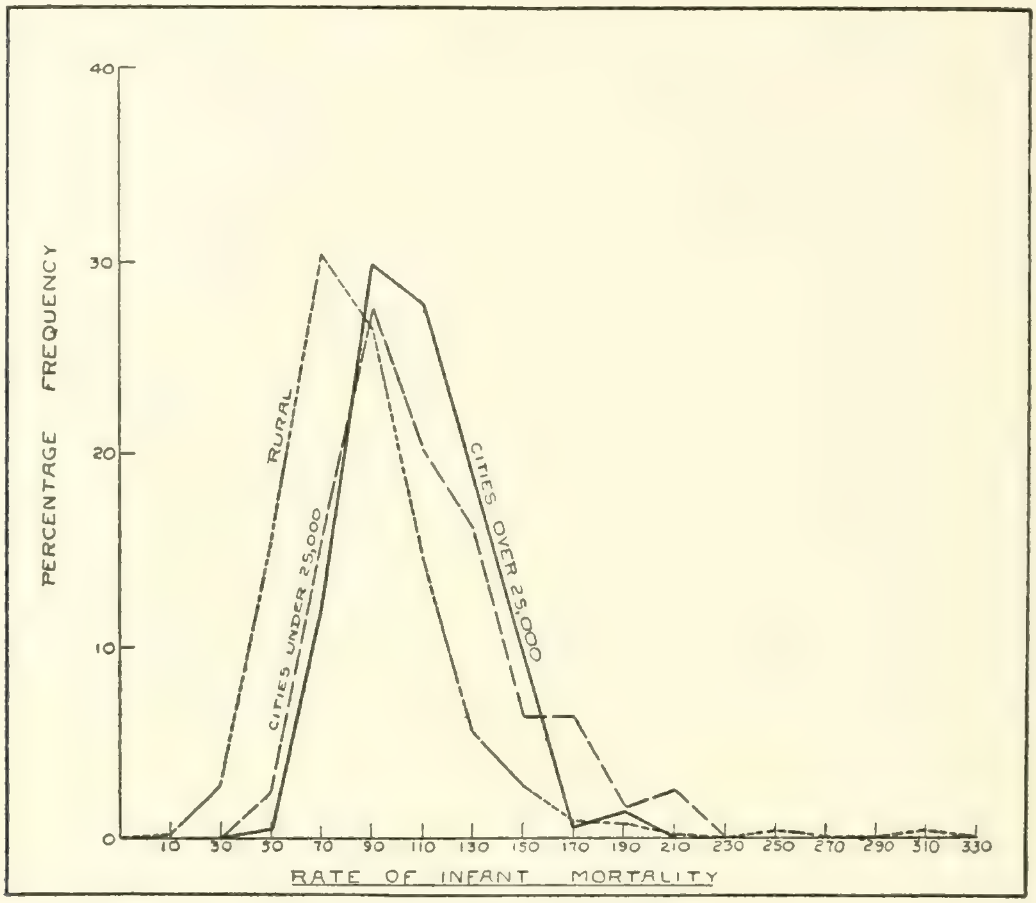

Fig. 1. Frequency polygons showing variation in the rate of infant mortality in 1918 for the total population of (a) cities of 25,000 and over, (b) cities of under 25,000, and (c) rural counties.

This diagram is fairly representative of all the distributions.

The most striking immediately observable feature of these distributions is the great range of variation which they exhibit. For example, in 1918 the 236 cities of under 25,000 inhabitants showed infant mortality rates ranging all the way from the class 40-59 deaths per thousand births to the class 300-319 deaths per thousand births. The range of variation is even greater than this in the case of the distributions for the colored population. These extraordinarily large ranges of variation demonstrate perhaps more clearly than could be done in any other way the opportunity which exists for effective administrative control and reduction of infant mortality. If there are communities, as there are in plenty, showing infant mortality rates under 100 deaths per thousand births it suggests at once that it is possible if the right measures are systematically and effectively applied to reduce the infant mortality in those other communities showing very high rates to something like the level of these at present more fortunate communities.

TABLE I.

Constants of variation in rate of infant mortality (deaths under 1 per 1,000 births)

Group	Mean[2]	Median[2]	Standard deviation[2]	Skewness
Cities over 25,000[1], Total, 1915	104.49 ± 1.78	102.76	26.14 ± 1.26	+.3148
" " " " 1916	102.53 ± 1.67	103.24	24.69 ± 1.18	—.0786
" " " " 1917	99.58 ± 1.32	98.00	23.45 ± .93	+.2455
" " " " 1918	107.78 ± 1.41	105.50	25.07 ± 1.00	+.3237
Cities under 25,000[1], Total, 1915	100.98 ± 1.68	97.95	30.81 ± 1.18	+.1934
" " " " 1916	104.23 ± 1.75	101.03	32.38 ± 1.24	+.2217
" " " " 1917	99.24 ± 1.32	94.74	29.94 ± .93	+.4840
" " " " 1918	111.61 ± 1.66	104.17	37.78 ± 1.17	+.5625
Rural counties, Total, 1915	83.07 ± .85	79.54	23.95 ± .60	+.3204
" " " 1916	85.28 ± .90	82.15	25.94 ± .63	+.3536
" " " 1917	82.01 ± .52	78.96	25.71 ± .37	+.2833
" " " 1918	84.43 ± .57	80.97	28.40 ± .40	+.4328
Cities over 25,000[1], White, 1917	92.22 ± 2.02	92.14	15.60 ± 1.43	....
" " " " 1918	102.59 ± 2.00	99.23	15.42 ± 1.42	....
Cities under 25,000[1], White, 1917	98.46 ± 2.75	97.50	20.82 ± 1.95	....
" " " " 1918	114.62 ± 4.17	113.33	31.49 ± 2.95	....
Rural counties, White, 1917	86.21 ± 1.07	84.24	24.15 ± .76	+.1799
" " " 1918	85.90 ± 1.27	83.75	28.90 ± .90	+.2802
Cities over 25,000[1], Colored, 1917	202.59 ± 8.88	194.00	68.45 ± 6.28	....
" " " " 1918	216.67 ± 11.15	214.00	85.87 ± 7.88	....
Cities under 25,000[1], Colored, 1917	213.08 ± 9.92	228.00	74.96 ± 7.01	....
" " " " 1918	217.69 ± 11.46	225.00	86.65 ± 8.10	....
Rural counties, Colored, 1917	134.76 ± 2.55	127.25	57.37 ± 1.80	+.4984
" " " 1918	147.26 ± 2.92	134.59	66.15 ± 2.06	+.5819

[1] In 1910.

[2] In concrete units, *i. e.*, rate of deaths under 1 per 1,000 births.

In Table I are presented the chief physical constants[4] of the distributions of variation in infant mortality. These constants have been determined by the method of moments from the original raw data.[5]

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[4] For a very brief and summarized introduction to the modern mathematical treatment of frequency curves see Pearson, K., "Tables for Statisticians and Biometricians," 1914, pp. lx to lxx. References to the basic literature on the subject will be found there.

[5] I am greatly indebted to my assistant, Mrs. Charmian Howell, for aid in the arithmetical work of this paper.

The constants tabled are:

1. The arithmetic mean.
2. The median. This measures the value above and below which exactly half of the variates occur.
3. The standard deviation. This constant measures in absolute units the degree of "scatter" or variation exhibited by the distribution.
4. The skewness. This constant measures the degree of asymmetry of a frequency distribution. If a distribution is perfectly symmetrical on both sides of the mean so that if folded over upon the mean as an axis the two limbs would exactly coincide, the value of the skewness is zero.

From the data presented in Table I. the following points are to be noted:

1. There is no certainly significant decline in the mean value of the rate of infant mortality during the four years covered by these statistics in any of the demographic units considered.

2. In 1918 there was a general tendency towards an increase in the mean rate of mortality over that which obtained in 1917. This increase is unquestionably to be attributed to the influenza epidemic of the autumn and winter of 1918. A careful examination of the rates by months will convince one that the mortality of infants increased very materially during the period of the epidemic. Whether this increased number of deaths was truly to be charged to influenza does not concern us here. The important fact is that the rate of infant mortality markedly increased coincidently with the existence of the epidemic. It is noteworthy that this increase in the infant mortality rate in 1918 is practically confined entirely to the cities. The rural counties, whether for white or colored or total population, show little or no change in 1918 as compared with 1917.

3. There is no unequivocal difference in the mean rates of infant mortality in the larger as compared with the smaller cities. Considering the largest differences in mean rates for total populations in cities of 25,000 and over as compared with cities of under 25,000 there is no difference which is as much as even three times its probable error.

4. The mean rates of infant mortality are notably smaller in the rural than in the urban areas. This fact has, of course, long been well known. The first writer on vital statistics, in the sense in which we now understand that subject, Captain John Graunt,

more than 250 years ago pointed out that rural communities exhibited generally a lower rate of mortality than urban communities. The difference between urban and rural rates of infant mortality is reflected just as clearly in the high absolute rates of the colored population as it is in the lower rates of the white population.

5. The mean rates of infant mortality are, roughly speaking, something like twice as high for the colored population as for the white population in each of the demographic units considered, and at all times. This again is a fact in general well known, but here we have precise figures on the point, with probable errors, which show definitely how tremendously poorer the negro baby's chances of surviving the first year of life are than the white baby's.

6. The cities of over 25,000 exhibit distinctly less variation in respect of infant mortality than do either the smaller cities (under 25,000) or the rural counties. The smaller cities and the rural counties exhibit about the same degree of variation relative to their means, but absolutely, in terms of standard deviation, the rural counties show less variability than the cities under 25,000. The colored distributions exhibit a much higher degree of variation in respect of infant mortality however measured, whether absolute or relative, than do the white populations. In general, it may fairly be assumed that the greater the variation exhibited by a given class of the community in respect of infant mortality, the greater the chance of effective control and reduction of the average infant mortality by administrative measures. There can be no question that there is no field which offers so great opportunities in this direction as the colored population.

7. The skewness is seen to be positive in sign in every case but one. In that case (1916, cities over 25,000 total) the skewness is not significant in comparison with its probable error. With this exception the curves tend to tail off more gradually and farther towards the right end than towards the left end of the range. In other words, the rate of infant mortality in these different American demographic units tends generally to distribute itself in a substantially unsymmetrical fashion about the mean, extremely high rates occurring more frequently than correspondingly low rates. This fact might perhaps be taken to indicate that the task confronting the administrative control of infant mortality in the United States, and

yet to be accomplished, is even greater than what has already been accomplished in the past, great and worthy of commendation as that is.

Data on the Limitations to Administrative Control of Infant Mortality.

We have seen that there is a high degree of variation in the rate of infant mortality as we pass from community to community. Some communities have infant mortality rates several times higher than those prevailing in other communities of the same size. This creates the presumption at once that proper administrative activity might reduce the rates of these abnormally high communities to a level commensurate with those found in the lower group. It is the purpose of this section of the paper to examine this presumption critically.

At the outstart it is evident that there are some causes of infant mortality which are, in their very nature, beyond hope of effective practical human control. Thus, children born with marked congenital hydrocephalus will presently die, in spite of anything the health officer can do, no matter how active and intelligent he may be. There are other causes of death falling in essentially the same category in this respect.

Not as any final or dogmatic settlement of the matter, but rather as a tentative first approximation made for the purpose of seeing whether any suggestive lead may appear, I have ventured to attempt to classify the principal causes of mortality in the first year of life into two groups. The first of these groups aims to include those important causes of infant mortality which are either (a) actually now effectively controlled by the efforts of health officials, either directly, or indirectly through general sanitary and hygienic improvements, or (b) are obviously capable theoretically of control and amelioration if sufficient pains be taken. The second group aims to include those causes of infant deaths which are either (a) in the nature of the case, out of the range of effective practical, *direct* control or amelioration, or (b) are not in fact now controlled in any appreciable degree. Let us see what such a classification, to a first approximation, looks like.

Tentative Classification of Principal Causes of Infant Mortality.

A. Causes of death actually now well controlled, or capable theoretically of direct control in greater or less degree:
- Measles
- Scarlet fever
- Whooping cough
- Diphtheria and croup
- Dysentery
- Erysipelas
- Tetanus
- Meningitis
- Convulsions
- Acute bronchitis
- Pneumonia
- Bronchopneumonia
- Diseases of the stomach
- Diarrhea and enteritis
- External causes

B. Causes of death not controlled
- Tuberculosis of the lungs
- Tuberculous meningitis
- Other forms of tuberculosis
- Syphilis
- Organic diseases of the heart
- Malformations
- Premature birth
- Congenital debility
- Injuries at birth

One realizes that it is a bold thing even to set down such a classification as the above. It is certain to stir up the rancor of extremists in both directions. But extremists are nearly always wrong. Calm and unprejudiced persons will admit that some such classification as that here attempted is possible. Perhaps some further discussion of this classification may make clearer its point of view, and may win at least that measure of agreement with it which will at least permit the consideration of the discussion of its consequences which follows.

Taking column A first, presumably no competent health official would deny that the first diseases in the list (scarlet fever, whooping cough, diphtheria and croup, and dysentery) have been, can be, and are in greater or less degree effectively controlled in respect both of their incidence and their mortality. With this same group clearly belongs also diarrhea and enteritis, and convulsions, on the justifiable assumption that in the vast majority of cases convulsions in infants are consequent upon violent enteric infections, which clearly belong in the controllable class. Diseases of the stomach, as causes of death *under one year of age,* again in the vast majority of cases undoubtedly mean infection—filth diseases in short—which come in the same category, so far as concerns control, as diarrhea and enteritis. Regarding the rest of the diseases in the A group (erysipelas, tetanus, meningitis, acute bronchitis, pneumonia, bronchopneumonia, and external causes) the point of view of which led to their inclusion here is as follows: If the environmental conditions surrounding the infant in the community and in the home, and the care given it, were made as favorable as they might be made, and actually are in the homes of the hygienically intelligent well-to-do, the death rate from each of these causes would be enormously reduced relatively in comparison with what it actually is. As a matter of fact visiting child welfare nurses are doing a mighty work in just this direction in many communities. They teach parents how to care for their infants, protect them from these infections, and nurse them to a non-fatal issue in many cases if they do get infected.[6]

Now for the B column. The first three items are the various forms of tuberculosis. The fanatic will no doubt promptly assert that nothing is so easily and readily controllable as these. But let us make haste slowly and remember certain things: First, that we are here talking about *deaths* under one year of age, that is *fatal* tuberculosis in the first months of life; and second, that our classification premises, in specific and stated terms, *direct control,* that is control through agencies acting directly upon the infant or his environment. *Theoretically* it is possible to reduce materially the mortality under 1 from tuberculosis. If every child born to tuberculous parents was instantly and ruthlessly removed from the home from the moment of birth, and reared in an environment where no contact with tubercle bacilli was possible, unquestionably enormously fewer infants would develop tuberculosis in the first year of life than now do. A recent paper by Bernard and Debre[7] furnished an instructive example, showing in a single case how a child removed at 1½ months from its tuberculous mother, threw off completely its own tuberculosis. But *practically* it is perfectly clear that neither in the past has there been, nor in the present is there, nor probably for some time in the future will there be rigid isolation of offspring from tuberculous parents to an extent or degree sufficient to influence the infant mortality from tuberculosis, in the entire Registration Area of the United States, by as much as one unit of the death rate. The mortality from tuberculosis under 1 year of age has to be sure declined during the past 40 or 50 years but no more rapidly than the general curve of tubercular mortality at all ages. But many persons fail to find any evidence that control measures have had anything to do in bringing down the general tuberculosis death rate. In this connection a recent paper by Given[8] on the influence of administrative or control measures upon the course of tuberculous mortality in general is interesting. He says: "Statistics show us that, in spite of all that has been said and done for the prevention of tuberculosis, our efforts in regard to pulmonary tuberculosis have not been attended with the anticipated success. The decline in mortality from this cause dates from 1838, and has continued steadily ever since down to 1913. Koch's discovery of the tubercle bacillus in 1882 does not appear to have affected it in any way."

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[6] No one who knows at first hand what child-welfare public health nursing is actually accomplishing in these directions will question the putting of these diseases in the controllable column. Their mortality rate can be materially reduced if communities will take the trouble to go intelligently about it.

[7] Bernard L., and Debre, R. Bull. Soc. Med. des Hosp. T. 44, p. 1658. 1920. Rev. in Jour. Am. Med. Assoc. March 19, 1921, p. 824. See also paper in the present volume of these Transactions, on "Prevalence and Management of Tuberculosis in Infancy," by T. C. Hempelmann, p.

I know of no evidence that anything now being done is sensibly influencing the rate of mortality from tuberculosis in infants under 1. Some individual physicians may have been particularly successful with a small number of tuberculous babies under his care. But statistically it means little in the toll of 2,501 deaths under 1 which tuberculosis is recorded to have taken in the Registration Area of the United States in 1918. Actually if the truth were known the total would be much larger even than this.

About fatal congenital organic diseases of the heart, congenital malformations grave enough to be fatal in the first year of life, and fatal congenital debility there will probably be no dispute. The mortality from fatal congenital syphilis is again, like tuberculosis, *theoretically* controllable.[9] But *practically* and in fact, is it controlled? The writer feels extremely doubtful about it.

Regarding premature birth, and injuries at birth, much the same reasoning applies, but with the additional consideration that presumably intelligent prenatal education of the mothers and improvement of prenatal environmental conditions would reduce these mortality rates in some unknown, but probably not large degree. Actually, however, there is no tangible evidence that these causes of death are in effect administratively controlled in any appreciable degree in this country at this time.

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[8] Given, D. H. C. Some deductions from the statistics on the prevention of pulmonary tuberculosis. Bull. Med. Jour. Feb. 12, 1921, pp. 225-226.

[9] Cf. Jeans, P. C. Syphilis and Infant Mortality. Trans. Am. Assoc. for Study and Prev. Inf. Mortality. Vol. IX, p. 155.

Finally it should be said that one occasionally important cause of infant mortality is omitted entirely from the classification. This is influenza. The reason for the omission is simply that the statistical discussion which follows is based upon 1918 mortality figures

TABLE II—Showing the deaths under one year of age per 1,000 living births for (A) controllable, and (B) non-controlled causes of death in certain American cities of 100,000 population or over in 1910.

City	Births in 1918	Deaths under one year				
		A. From causes controllable in some degree	A. Rate of controllable deaths	B. From causes not controlled	B. Rate of not controlled deaths	Rate per 1,000 births from all causes
Bridgeport	4,910	226	46	224	46	100
New Haven	4,869	190	39	200	41	90
Washington	8,162	399	49	450	55	112
Indianapolis	6,196	270	44	269	44	93
Louisville	4,368	239	55	210	48	112
Baltimore	15,143	1,225	81	847	56	149
Boston	20,062	1,092	54	984	49	115
Cambridge	2,672	144	54	111	42	107
Fall River	3,646	403	111	183	50	180
Lowell	3,286	302	92	180	55	159
Worcester	5,238	212	40	248	47	97
Detroit	27,036	1,296	48	1,199	44	100
Grand Rapids	2,836	110	39	119	42	86
Minneapolis	8,704	198	23	358	41	73
St. Paul	5,155	160	31	135	26	87
Albany	2,153	96	45	122	57	115
Buffalo	13,989	866	62	653	47	121
Bronx Borough	16,763	496	30	669	40	75
Brooklyn Borough	49,515	2,232	45	1,889	38	90
Manhattan Borough	59,227	2,855	48	2,456	41	97
Queens Borough	9,467	389	41	417	44	93
Richmond Borough	2,677	113	42	139	52	106
Rochester	6,855	283	41	276	40	92
Syracuse	4,352	265	61	206	47	119
Cincinnati	7,913	326	41	404	51	104
Cleveland	20,699	963	47	790	38	98
Columbus	4,464	163	37	255	57	101
Dayton	3,282	109	33	143	44	87
Toledo	5,524	186	34	270	49	94
Philadelphia	43,408	2,876	66	1,993	46	124
Pittsburgh	15,875	1,179	74	805	51	139
Scranton	3,139	263	84	141	45	141
Providence	6,384	342	54	352	55	123
Richmond, Va.	3,840	199	52	285	74	147
Seattle	5,910	93	16	218	37	61
Spokane	2,194	55	25	90	41	77
Milwaukee	11,090	574	52	488	44	106

and inasmuch as that was a year in which the influenza mortality was abnormally heavy owing to the epidemic it was thought that it would be unfair to the general relationships exhibited to include this epidemic mortality. Presumably normal endemic influenza should be in the A group, on the same reasoning as the pneumonias.

With so much of explanation as to the point of view of this classification let us examine some of its statistical consequences. These consequences I have tested in a preliminary way upon the birth and death data for certain large cities and the registration states in 1918. There were found to be 37 large cities included in both Birth and Death Registration Areas in that year, and 20 states. For each of these cities and states the births were taken from 1918 Birth Statistics and the deaths under one year of age according to causes from Table II of the 1918 Mortality Statistics. From these data the rates per thousand living births for all class A and all class B diseases were separately calculated. The results are set forth in Tables II and III.

TABLE III—Showing the deaths under one year of age per 1,000 living births for (A) controllable, and (B) non-controlled causes of death in twenty registration states.

State	Births in 1918	Deaths under one year				
		A. From causes controllable in some degree	A. Rate of controllable deaths	B. From causes not controlled	B. Rate of not controlled deaths	Rate per 1,000 births from all causes
Connecticut	36,971	1,755	47	1,723	47	107
Indiana	64,385	2,482	39	2,526	39	87
Kansas	39,117	1,163	30	1,522	39	80
Kentucky	62,338	2,325	37	2,328	37	93
Maine	16,798	670	40	743	44	101
Maryland	34,113	2,531	74	1,730	51	140
Massachusetts	95,640	5,284	55	4,324	45	113
Michigan	91,011	3,496	38	3,760	41	89
Minnesota	55,941	1,317	24	2,060	37	71
New Hampshire	9,642	451	47	499	52	113
New York	242,155	10,897	45	10,333	43	97
North Carolina	75,525	2,850	38	2,319	31	102
Ohio	124,586	5,029	40	5,206	42	94
Pennsylvania	220,170	14,506	66	10,295	47	129
Rhode Island	15,499	947	61	783	51	126
Utah	14,478	308	21	474	33	64
Vermont	7,507	258	34	343	46	93
Virginia	63,062	2,529	40	2,448	39	103
Washington	25,682	544	21	980	38	69
Wisconsin	60,867	1,854	30	2,334	38	79

In the last column of these tables the gross infant mortality rates from all causes of death have been inserted for comparison and to furnish the basis of certain discussions which will follow. It will be noted that the five boroughs of New York City have been treated as separate cities. This appears to be entirely justifiable, both on grounds of size, and of differentiation, any two of these boroughs being as much differentiated biologically and demographically as, for example, Minneapolis and St. Paul.

The first point which strikes one in examining Tables II and III is that in the group of causes of death subject to our classification (which includes in most cases, as will be seen, something over 90 per cent of all the mortality under one year of age) the controllable and uncontrolled causes are responsible for approximately an equal degree of mortality. In other words, it appears that if any degree of justification attaches to the classification here suggested, the infant mortality beyond present control by administrative measures is by no means a negligible fraction of the total infant mortality. On the contrary, it represents a substantial lower limit sensibly below which the health officer, no matter how zealous and intelligent his activities, may not hope to go at the present time.

If there is a substantial moiety of the existing infant mortality which is uncontrolled by administrative measures and is essentially unaffeected by the present or past application of such measures, we should expect that the rate of mortality represented by this moiety would vary but little from city to city or state to state. As we have seen, the main reason why this part of the total infant mortality is beyond control is because it depends upon fundamental biological factors inherent in the parents and the infants. Clearly if this is so, whatever variation appears in this portion of the total infant mortality rate as we pass from community to community must arise from some combination of two factors; of which the first and less important is pure chance, that is, variation arising from random sampling purely; and of which the second is differing racial and other biological characteristics of the populations of the several communities. We should expect the variation in the death rate from the class B group of causes to show very little variation as compared either with the variation in the rate from class

A causes or in the gross infant mortality rate from all causes. This *a priori* expectation is beautifully realized in the actual statistics.

TABLE IV—Frequency distributions of variation in rates of mortality under one per thousand births for (A) controllable, and (B) non-controlled causes.

Rate	Cities			States		
	A Causes	B Causes	All Causes	A Causes	B Causes	All Causes
15- 24	2	..	..	3	..	..
25- 34	5	1	..	3	2	..
35- 44	9	16	..	7	11	..
45- 54	12	13	..	3	7	..
55- 64	3	6	1	2	..	1
65- 74	2	1	1	2	..	2
75- 84	2	..	2	..	..	2
85- 94	1	..	9	..	..	5
95-104	..	..	7	..	..	4
105-114	1	..	5	..	..	3
115-124	..	..	6	..	..	..
125-134	..	..	..	..	..	2
135-144	..	..	2	..	..	1
145-154	..	..	2	..	..	..
155-164	..	..	1	..	..	..
165-174	..	..	..	..	..	..
175-184	..	..	1	..	..	..
Totals	37	37	37	20	20	20

TABLE V.

Variation constants from the distributions of Table IV.

Group	Mean	Median	Standard deviation
Cities, A, controllable causes............	49.46 ± 2.04	47.08	18.37 ± 1.44
Cities, B, non-controlled causes..........	47.30 ± .90	46.15	8.09 ± .63
Cities, all causes	107.84 ± 2.75	102.86	24.78 ± 1.94
States, A, controllable causes............	42.00 ± 2.17	40.71	14.41 ± 1.54
States, B, non-controlled causes..........	42.50 ± .83	42.27	5.52 ± .59
States, all causes	97.00 ± 3.03	95.00	20.07 ± 2.14

It is seen that the *class B causes of death, which are not practically capable at the present time of administrative control or amelioration, exhibit less than one-half as much variation in the rate of infant mortality for which they are responsible, as we pass from city to city or from state to state, as do the class A causes of death, which are capable of administrative control.* This relation is true however the variation is measured. This is a novel result, of interest from several points of view.

In the first place, the suggestion lies near at hand that if the class A causes of death, which are controllable, show such great variation relatively as they do, it must mark an approximately equal variability in the zeal, intelligence, and efficiency of the administrative health officials of these communities. Anyone at all familiar with the organization of municipal and state health departments in this country will find it extremely interesting to study in detail the entries of Tables II and III noting how the class A (controllable) and the "all causes" rates fluctuate up and down, while the class B (non-controlled) rates stay, with a very few exceptions, so extremely constant. One will observe, with great satisfaction what splendid work is being done in some communities in holding down to a low level the infant death rate from controllable causes. Table II forms a real justification of the faith that is in the public health official of vision. It shows that the infant mortality from controllable causes *can* be kept down to a low level, and is in some communities. In the following cities (17 out of 37) the rate of infant mortality from the controllable causes of class A is actually *lower* than the rate from the non-controlled causes (class B).

New Haven
Washington
Worcester
Grand Rapids
Minneapolis
Albany
Bronx Borough
Queens Borough
Richmond Borough
Cincinnati
Columbus
Dayton
Toledo
Providence
Richmond
Seattle
Spokane

These cities stand as examples of the fact that a considerable portion of the infant mortality rate can be controlled.

Summary.

This paper is a first biometric survey of the infant mortality statistics of the recently established Birth Registration Area. It is to be regarded as preliminary to certain analytical studies of the problem of infant mortality now in progress in this laboratory. The chief results of the paper are first to set forth and discuss some of the constants of variation in infant mortality in the different demographic units. This variation, which is large in amount, markedly and consistently skew in the positive direction, defines and throws into high relief the fundamental public health or administrative problem of infant mortality. Why do the communities having rates of infant mortality higher than the average occupy that position? Is it from causes capable of human control, or from causes beyond the possibility of such control? A special preliminary analysis of the data for cities of over 100,000, and the registration states indicates that causes of death capable of administrative control are chiefly responsible for the variation observed in the total infant mortality rate, while those causes of infant deaths which, for fundamental biological reasons, are not sensibly influenced or controlled by administrative measures, are a highly stable and constant factor from community to community, contributing little to the observed variability of the total infant mortality rate. In absolute terms, however, these causes of death not administratively controlled are responsible for roughly 40 per cent of the total infant mortality in the communities discussed.

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THE RELATIVE INFLUENCE OF THE CONSTITUTIONAL FACTOR IN THE ETIOLOGY OF TUBERCULOSIS[1]

RAYMOND PEARL

I. THE PROBLEM

The problem to which attention is directed in this paper is that of the etiology of clinically active tuberculosis. A few deny the existence of any such problem. Happy in that superlative self-esteem vouchsafed in full measure only to ardent worshippers at the shrine of *idola fori*, they would, if they could, stop all true research on the problems of tuberculosis, and give countenance only to such so called investigative activities as are guaranteed beforehand not to run counter in any particular to the plentiful exhibition of their special brand of eye-water, blatantly touted as the only true panacea for the ills tuberculosis engenders. Fortunately, all scientific students of tuberculosis realize keenly that the problem of the etiology of the disease is a real and important one. The case cannot be better put than it is in a recent paper by Lawrason Brown and his associates (1) where they say, "Our hope in publishing these experiments is that others may realize that the etiology of tuberculosis is not a closed book, but one that contains many disconcerting and confused pages that need to be rewritten."

The problem may with some precision be defined by a statement of the following well-known and thoroughly established facts:

1. Very few, if any, persons escape infection with tubercle bacilli at some time in their lives, especially if they live in large industrial cities. Fishberg (2), after reviewing the literature on the subject says, "No matter what the cause of death may have been, whether the persons knew that they had been tuberculous or not, between 50 and 100 per cent of people living in large cities show active, quiescent or healed tuberculous lesions in some organs of their bodies."

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[1] Papers from the Department of Biometry and Vital Statistics of the School of Hygiene and Public Health of the Johns Hopkins University, No. 20.

This paper was read before the North Atlantic Tuberculosis Conference at Richmond, Va., October 7, 1920, in a symposium on the general topic "The Basic Cause of Breakdown with Tuberculosis."

2. A much smaller number of persons than are known and can be proved to have been at some time or other infected with tubercle bacilli ever develop clinically recognizable tuberculosis.

3. Of the two moieties of the infected, those on the one hand who do, and those on the other hand who do not develop the disease in clinically active form, many can be readily shown to have lived under essentially or statistically the same environmental circumstances.

4. What factors determine the group into which a *particular* infected person shall fall, and what is the quantitative influence of each particular possible factor in making this determination?

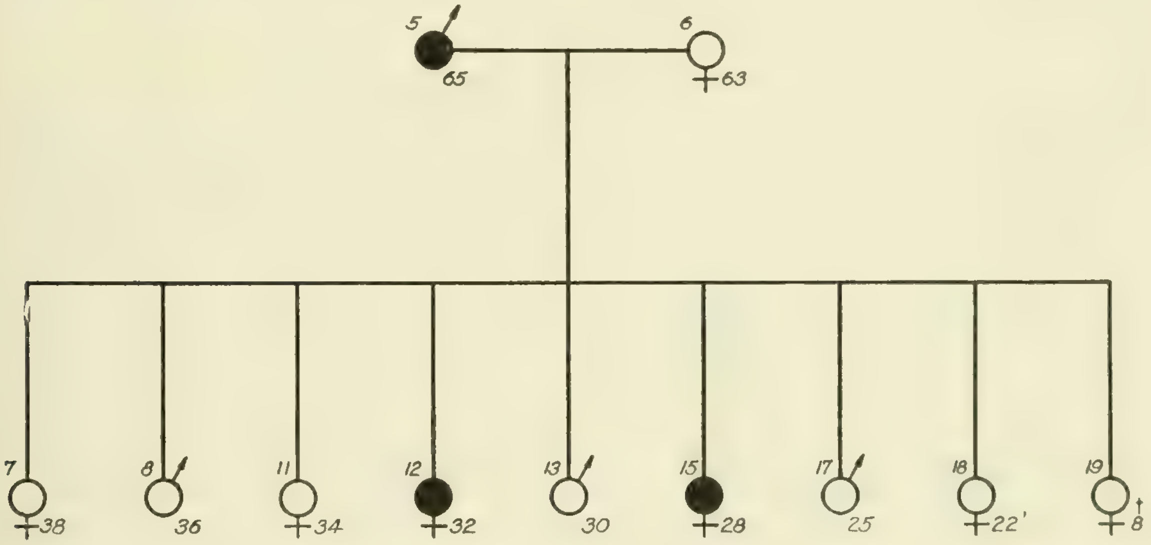

Fig. 1. Pedigree Chart of a Tuberculous Family

Where the body of the sex sign is solid the person has tuberculosis of the lungs; where it is open the person is not tuberculous. The numbers to the right of the bottom of each sex sign indicate the present age, or age at death of the person indicated. The numbers to the left of the top of the sex signs are the designating numbers (in lieu of names) of the indicated persons. + denotes that the person is dead.

This I conceive to be the fundamental and essential problem of the etiology of clinically active tuberculosis.

The problem can be still more precisely visualized by the consideration of a particular family taken from one of the pedigrees worked up in my laboratory.

The time relations of contact of the children in the tuberculous family, whose pedigree is given in figure 1, with their tuberculous father, and with each other, are given in summary form in table 1.

The essential facts regarding this family are as follows: The father, individual no. 5, is a laboring man who has always been addicted to the use of alcohol in considerable quantities. In 1886, when he was thirty-two years old, he developed clinically active pulmonary tuberculosis and had frequent hemorrhages. He was in this condition when child no. 12, was born. The firstborn daughter, no. 7, has always been at home with the family down to the present time, having thus lived thirty-three years in close contact with an active case. She is by occupation a seamstress. She has never developed any indications of tuberculosis. The first son, individual no. 8, was born in 1883 and lived at home in the same household with his father until he was twenty-eight years old (twenty-five years familial contact). He has never developed any symptoms of tuberculosis. The next child, no. 11, was born in 1885 and lived at home with the family, in daily close contact with her tuberculous father for twenty years. She has never developed any symptoms of tuberculosis. The next child was born in 1887. In 1913, when she was twenty-six years old, she developed clinically active pulmonary tuberculosis after having lived twenty-six years in the same household and in close contact with her tuberculous father. Individual 13 was born in 1889 and lived for the first twenty-five years of his life in the same household with the tuberculous father. He has never shown any symptoms of tuberculosis. Individual no. 16 was born in 1891, and in 1912, when she was twenty-one years old, developed clinically active pulmonary tuberculosis. She had lived all this time, and still does, in the same family with her father. Individual no. 17 was born in 1894 and has

TABLE 1

Contact relations involving individuals in pedigree chart

INDIVIDUAL NUMBER	BORN	BECAME TUBERCULOUS	YEARS LIVED IN CLOSE CONTACT IN SAME HOUSEHOLD WITH CASES BEFORE OR WITHOUT ACQUIRING TUBERCULOSIS
5	1854	1886	
6	1856		33
7	1881		33
8	1883		25
11	1885		20
12	1887	1913	26
13	1889		25
16	1891	1912	21
17	1894		24
18	1897		22
19	1880		8

always lived with the family until he was drafted and went into the American Expeditionary Force and to France. Since his discharge he is again living with the family. He has never developed any symptoms of tuberculosis, although he has lived for roughly twenty-four years of his life in close daily contact with his father, and also with his two sisters, since the time when they became tuberculous. Individual no. 18 was born in 1897 and has always lived in the same household with her father and the rest of the family. She is now twenty-two years old and thus has had close intimate household contact for twenty-two years with active tuberculous patients. Individual no. 19 was born in 1880, and died when eight years old, probably of acute nephritis, but certainly not of tuberculosis. Individual no. 6, consort of the tuberculous father, has lived in close and intimate household contact with him for the thirty-three years since he first gave evidence of clinically active tuberculosis. She has never shown any symptoms of any tuberculous disease.

Putting all the facts in regard to this family together what do we see? Out of eight living children, all of whom lived together in the same environment, and had the same food, for twenty or more years, two have developed clinically active tuberculosis, while the other six have shown no signs whatever of the disease. All have been in close and intimate personal family contact with their father, who has, as our careful investigation of the family life and medical history indicates, been an open tuberculous patient during the major portion of that time, unable to do any work for most of the time, with hemorrhages recurring at irregular but fairly frequent intervals. Furthermore, individuals nos. 12, 16, and 18 have for many years slept together in the same room. Nos. 12 and 16 have been tuberculous for six and seven years respectively, while 18 has never shown any symptoms of the disease.

It is to be presumed that every member of this family was long ago infected with tubercle bacilli. If we attribute breakdown to poor diet, or to bad housing conditions, it is difficult to see why the disease should have flared up in clinically active form in only two out of eight children who have lived in this same environment throughout the period. All have been subjected to substantially the same degree of environmental and economic pressure. They have all had to work hard, because the father has contributed very little to the support of the family in all these years.

Such a case as this, which could be duplicated over and over from our family records, throws into sharp relief what I conceive to be the essential problem of the etiology of tuberculosis. Out of an equally infected group of individuals, equally in close contact with active and open tuberculosis, and living under identical environmental conditions, some individuals in the group develop clinically active tuberculosis while others never do. What is the reason for the differentiation? This is the kernel, as it seems to me, of the problem of the etiology of tuberculosis.

The investigation on which this family pedigree which we have been discussing is based was made in 1919. It may be objected that all of the children, 7, 8, 11, 13, 17, and 18, will presently develop tuberculosis and die of it. No one can assert, of course, that this will not happen because it is an event which belongs in the future. I conceive it to be improbable, however, that such an event will occur. The force of untoward environmental pressure on these individuals during the past twenty years has been about as great as it could be, and certainly greater than it is likely to be in the future.

Another objection which might be conceivably raised to the illustration is that neither individual 5, 12, nor 16 has tuberculosis. The answer to this is that they have repeatedly had diagnosis and treatment of the disease in sanatoria and dispensaries. If they are not victims of the disease no one is.

I wish it to be quite clearly understood that this pedigree is introduced here only as an illustration of the problem, and not in any sense, taken by itself, as evidence of the influence of the hereditary factor in the etiology of the disease. It obviously suggests such a factor, but suggestion and demonstration are usually a long way apart. It does illustrate with great clearness and precision the nature of the problem facing one who would account for the etiology of clinically active tuberculosis.

II. HOW SHALL THE PROBLEM BE ATTACKED?

It is obvious on purely *a priori* grounds that in a broad sense the cause of the outbreak of clinically active tuberculosis in one moiety of a group of infected individuals and not in the other moiety must fall into one of the three following categories:

1. Environmental. There are significant differences in the environmental forces, using the term in the broadest sense to include biological as well as physical environment, impinging upon the two moieties, these differences being of sufficient magnitude alone to determine the observed difference in susceptibility to tuberculosis.

2. Constitutional. There are significant differences, of a germinal, hereditary character, in the innate biological constitutions of the individuals composing the two groups, and these differences alone determine the differentiation in respect of outbreak of active tuberculosis.

3. Both. The two groups differ in both environmental and constitutional respects, neither factor alone being ordinarily determinative of tuberculous breakdown.

The first of the enumerated views is the popular one. It opens large possibilities for altruistic enterprise. I judge from what I hear and read that there are still extant naïve individuals who quite honestly believe that if everyone could be induced and enabled to drink two quarts of milk a day and sleep with his windows open, tuberculosis—certainly pulmonary tuberculosis— would disappear from the face of the earth by the middle of next June at the very latest. If only these good souls were right, how happy we all should be!

The second view is extremely unpopular. Any doctrine which implies that the constitutional weaknesses, whether patent or hidden, of the father might be visited upon the sons stirs up no furious enthusiasm in this day and age. The person who has, or had, a number of tuberculous near relatives finds himself particularly antipathetic to this view. If indulged in, what becomes of one's hope in the two quarts of milk or the open window?

The relative popularity of a viewpoint happily has nothing to do with its validity. Extremists in any direction are usually wrong. It is altogether improbable that either environment or hereditary constitution *alone* determines the differential incidence of active tuberculosis. It is much more likely that always both factors are involved. The task is, as I conceive it, not to answer the question "Is it environment?" or "Is it heredity?" but rather to measure, with the greatest attainable precision the relative influence of each factor in determining the result.

In earlier days constitution was generally regarded by medical men as playing a very important rôle in the etiology of tuberculosis. The most comprehensive and critical discussion of the older literature on this point has been furnished by Bulloch and Greenwood (3).

There are only a few chief lines yet thought of by which one can get quantitative evidence as to the relative importance of the hereditary factor in the etiology of tuberculosis. We may direct our attention briefly to some of these different lines of evidence, it being understood, of course, that in the limitations of space which are imposed upon this paper, it will be impossible to deal with the matter in more than the most sketchy fashion. In other words, what we shall try to do is to present something about the *kinds* of evidence, rather than the amount of each kind which may be brought to bear in any consideration of the problem.

1. Direct evidence as to the inheritance of the tuberculous diathesis. Here substantially the only studies up to the present time which are deserving of serious scientific consideration are those made in Pearson's (4) laboratory, or under his direction. Pearson has attacked the problem of the relative influence of heredity and environment in the etiology of tuberculosis from a number of different angles. In this section we shall consider only one, namely, that side of his work in which he attempts to measure directly the degree of inheritance of the tuberculous diathesis from parent to offspring. This is done by the method of correlation. The coefficient of correlation between parent and offspring in respect of presence or absence of pulmonary tuberculosis is determined by well-known methods, and it is assumed that the correlation which is found is due to inheritance. Unfortunately, the series dealt with were not as large as would be desirable from a statistical standpoint. The general results attained by Pearson for the correlation between parent and offspring in respect to existence of pulmonary tuberculosis are shown in table 2.

TABLE 2

Coefficients of correlation between parent and offspring

CHARACTER		CORRELATION	AUTHORITY	DATA
Physical	Stature	0.51	Pearson and Lee	Pearson's F. R.
	Span	0.46	Pearson and Lee	Pearson's F. R.
	Forearm	0.42	Pearson and Lee	Pearson's F. R.
	Eye color	0.50	Pearson	Galton's F. R.
Pathological	Deaf-mutism	0.54	Schuster	Dr. Fay's
	Insanity	0.53	Heron	Dr. Urquhart's
	Insanity	0.47	Goring	Convict prisons
	Phthisis	0.50	Pearson	Dr. Rivers's
	Phthisis	0.50	Goring	Convict prisons

From this table it will be seen that, on the face of the facts, the indication is that the tuberculous diathesis is inherited in about the same degree from parent to offspring as such physical characters as stature, forearm length, eye-color, etc. The contention of the environmentalists and infectionists, however, upon the appearance of these results, was at once that such correlations might well arise solely by familial infection, rather than true inheritance.

Pearson has attempted in a number of ingenious ways to meet this point. Whether one considers that he has successfully done so or not depends, one gathers from the literature, upon the nature and degree of one's prior prejudices in the matter. It will, however, pay us to look at a few of the facts which Pearson has brought forth. If the explanation of the observed correlation between parent and offspring in respect of tuberculosis is familial infection rather than similarity of biological constitution, then it would seem obvious that the correlation between husband and wife in respect of tuberculosis ought to be of something approaching the same order of magnitude as that shown for parent and offspring. If the reason why a tuberculous man's children have tuberculosis in greater proportion than the disease occurs in the general population, is because he has infected them as a result of close familial contact, by the same token his wife ought to be tuberculous. The only quantitative difference, which might possibly be expected, would be such as arose from the fact that when a consort came in contact with the familial tuberculous infection, he or she would be at a more advanced age. It might be contended that at such age the individual would perhaps be better able to resist such infection than the children born in the family and subjected to this immediate and massive source of infection from the earliest ages. Leaving aside any attempt at explanation, the fact is that the resemblance between husband and wife in respect of tuberculosis is as follows (quoting from Pearson):

		Coefficient of correlation
Husband and Wife	All poor	−0.01
Husband and Wife	Prosperous poor	+0.16
Husband and Wife (Pearson)	Middle classes	+0.24
Husband and Wife (Dr. Williams)	Professional classes	+0.28

It would thus appear, either that like mates with like more commonly in the more intellectual classes, or that infection is more likely to occur in middle class than in poor households. I think there is not the least doubt that much of the relatively small resemblance of husband to wife in the matter of phthisis is due to a selective influence and not to infection at all. This selection is largely an intellectual one and has no existence among the very poor.

Pearson went on to show from his family records, leaving entirely out of account the husbands and wives themselves, and determining the degree of assortative mating between tuberculous *stocks*, that the correlation worked out to 0.30, as high as in the extreme cases of mating with regard to pulmonary tuberculosis.

Now if we compare the correlation coefficients for parent and offspring as shown in table 2, with those for husband and wife just given, it would indicate that the parent is twice as dangerous to the offspring, if the source of the resemblance is familial infection, as the husband is to the wife. This, of course, common sense tells us is a highly improbable result. It tends to indicate that neither correlation is primarily to be explained on the basis of infection, but that the one is due to inheritance of the diathesis, and the other to assortative mating of tuberculous stocks.

The whole case, however, leaves one with the feeling that so far as the direct measurement of inheritance is concerned, we need more and better evidence than that which has been furnished by Pearson. We need, in the first place, more critically accurate original data, in which we shall have exact and direct information, in so far as it is possible to get it, obtained by trained field workers in the families, regarding such points as degree and duration of familial contact with open cases, as well as the more usual points upon which data are taken. At the same time, we need equally critical data from nontuberculous families collected in the same way by field workers in the families. Again it is not entirely clear from a methodological viewpoint that the correlation method is a sound one, taken by itself alone, for the measurement of the intensity of inheritance. The results got in this way need confirmation by some independent method of analysis before they can be unreservedly accepted at their face value. Pearson's was pioneer work, and it unquestionably strongly indicates that the hereditary factor is a highly important one in this disease. Only the reckless environmental fanatics will be disposed to neglect it, or to regard it as wholly worthless. The problem to be solved is, however, one of such vast importance to the human race, that before any sweeping conclusions are drawn on which administrative action is likely to be based, it is essential that much further investigation of the problem be made. Furthermore, any statistical or family history evidence on the subject should be checked and verified by direct experimental evidence in the laboratory, obtained from breeding operations with lower animals, as to the inheritance factor in the etiology of tuberculosis. It is a great satisfaction to know that investigations of this kind are now in progress. Their results will be eagerly awaited by all students of the problem.

In order to illustrate directly some of the difficulties which inhere in any attempt to make a thorough scientific investigation of the problem of the inheritance of the tuberculous diathesis in man, I should like to speak briefly about some of the work going on in my own laboratory. About a year and a half ago plans for a comprehensive and searching investigation of the problem of the etiology of clinically active tuberculosis were embarked upon.[2] While it was contemplated that the problem would be approached from many angles, and this is in fact being done, I wish to speak now of one phase of the work, namely, that which has to do with the obtaining and analyzing of data directly bearing upon the problem of inheritance of the diathesis. The plan of the work was to make a thorough study of the family histories of individuals afflicted with tuberculosis, and of individuals free from tuberculosis, both sets of individuals being taken from the same economic and social strata. "Family history" in this connection is used in the broadest possible sense to include not only information as to relatives direct and collateral, but also as many pertinent data as possible about the individual life histories and habits of these individuals.

The work is in far too early a stage now to make any statement of results whatever, nor do I care at this time to go into any discussion of the details about either the comprehensiveness or the thoroughness of the records which we are accumulating. I may merely say in passing that, so far as I am acquainted with the facts, I know of no data for the study of the etiology of this or any other disease which begin to approach these in respect of either comprehensiveness, detail, or accuracy. Our ideals in these respects are set very high, and in consequence a number of years must still elapse before a sufficient amount of material can have been collected for the kind of analysis which must be made. Here I wish to use a little of the material, only for the purpose of illustrating the difficulties which beset research upon this problem.

If heredity is a factor of importance in the etiology of clinically active tuberculosis it would be reasonable to expect that a tuberculous individual would have a larger proportion of his or her blood relatives, both direct and collateral, and in ascending and descending generations from the individual, tuberculous than would a person who was not tuberculous. Or, to put the matter in another way, suppose we stopped the first man or woman we chance to meet on the street and ascertained by appropriate methods whether that person was or was not tuberculous, and at the same time made detailed inquiries as to his or her blood relatives. Should we be justified in laying a wager, if the individual proves to be tuberculous, that a larger percentage of his relatives will be also tuberculous than if he himself were nontuberculous, and what if any odds could we give in such a wager? In table 3 I have collected together the data on this point from 57 family histories which we have been able to complete to date, involving something over 5000 blood relatives of the 57 subjects of these family histories. Of these subjects, from each of which a detailed history starts, 38 are tuberculous, and 19 are nontuberculous. Each group may be regarded as a random sample of the working class population of Baltimore, the only differential factor in the selection being that in the one case the individual with whom a history started was tuberculous, and in the other case not.

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[2] During the past year this investigation has been supported by a grant from the National Tuberculosis Association.

TABLE 3

Showing the frequency of occurrence of tuberculosis among the blood relatives of (a) tuberculous persons and (b) nontuberculous persons

(Figures based upon the family histories of 38 tuberculous and 19 nontuberculous subjects)

GENERATION AND GROUP RELATIVE TO SUBJECT OF HISTORY	NUMBER OF BLOOD RELATIVES WHO ARE			PER CENT TUBERCULOUS
	1. Tuberculous	2. Nontuberculous	Total	
Same generation				
a. Tuberculous subject	96	876	972	9.9
b. Nontuberculous subject	5	979	984	0.5
Parental generation				
a. Tuberculous subject	42	430	472	8.9
b. Nontuberculous subject	14	749	763	1.8
Grandparental generation				
a. Tuberculous subject	7	236	243	2.9
b. Nontuberculous subject	8	298	306	2.6
Great grandparental generation				
a. Tuberculous subject	3	30	33	9.1
b. Nontuberculous subject	1	86	87	1.1
Child generation				
a. Tuberculous subject	43	972	1015	4.2
b. Nontuberculous subject	0	212	212	0.0
All generations				
a. Tuberculous subject	191	2544	2735	7.0
b. Nontuberculous subject	28	2324	2352	1.2

Five generations are included in the table. There are: First, the generation to which the subject of the inquiry himself belongs: this is the generation in which his blood relations are brothers, sisters, or cousins; second, the parental generation, that is, the generation to which the subject's father and mother, and his uncles and aunts belong; third, the grandparental generation; and fourth, the great grandparental generation. The "child generation" is the generation to which the subject's children, and his nephews and nieces belong. Relatives by marriage solely, of course, are not included. Only those persons are included who have a biological or "blood" kinship to the subject in some degree or other. But all *degrees* of kinship, collateral and direct, near and remote, are included.

The net result is striking. Taking all the generations together we see that in these statistics a tuberculous person has 7 per cent of his or her blood relatives tuberculous, whereas a nontuberculous person, chosen at random, has only 1.2 per cent of his or her blood relatives tuberculous, the absolute numbers involved in the two samples being approximately the same. In other words, in so far as this material may be regarded as typical, we can assert that a tuberculous person chosen at random from the working class population will have nearly six times as many blood relatives tuberculous as will a nontuberculous person taken at random from the same population. We note that the same kind of difference appears in each generation. The difference is insignificantly small in amount in the grandparental generation. This must be attributed, I think, merely to random sampling. I judge that with larger numbers, this generation would pass into the same class as the others.

One other point which needs explanation, lest an erroneous conclusion be inadvertently drawn from table 3, appears in the figures for the child generation. It will be noticed that whereas the histories of the 38 tuberculous subjects yield 1015 blood relatives in the child generation (F_1 generation in current genetic usage) the 19 histories of nontuberculous subjects yield but 212 individuals in the same generation. This does *not* mean, as might be supposed, a reduced fertility in the nontuberculous as compared with the tuberculous. The difference arises merely from the fact that the method of selection of subjects upon which to start histories is such as to give a considerably lower average age of *subject* in the nontuberculous group. Naturally they would have fewer offspring in the F_1 generation. Briefly the method of getting cases is this: Tuberculous subjects are taken at random from the list of active cases registered in the Tuberculosis Bureau of the Baltimore Health Department. Nontuberculous subjects are taken at random from cases which have passed through the Juvenile Court of Baltimore at one time or another. The individuals are naturally, on the average, younger in the latter case than in the former.

An inexperienced person, not aware of the pitfalls which strew the pathway of the investigator of tuberculosis might think that table 3 told the whole story; that it in short proved the case for the inheritance of the disease. But it will pay us to look a little further into the matter before jumping to this alluring conclusion. Amongst other data which we have collected in this work we have included elaborate and detailed information wherever possible as to the duration and extent of personal contact of individuals in the family history with persons in an active "open" tuberculous condition, whether in the family home or outside. Of course it is impossible to get this information accurately for every individual in a pedigree, and consequently the number of individuals appearing in the following tables is smaller than in table 3. Let us examine the data obtained from such inquiries. The material is exhibited in tables 4 and 5. In this table the headings have the following meanings:

1. *Close contact* means that the enumerated offspring, whether tuberculous or not, were *known* to have been in close and frequent contact with an active case or cases of tuberculosis in their relatives or elsewhere. In case of the tuberculous offspring this contact existed for some time *before* they, the offspring, became themselves tuberculous.

2. *No close contact* means that the enumerated offspring, whether tuberculous or not, were known *not* to have been in close and frequent contact with an active case of tuberculosis among their relatives, and there is no evidence that they were in such contact with active cases among other persons.

Table 4 gives the raw material so far available.

The data of table 4 are summarized in percentage form in table 5. Inasmuch as the number of cases for some of the ancestral combinations is small, we have ventured to combine certain of the rubrics of table 4. It will be noted that even in this small beginning of the contemplated investigation we have included in table 4, 690 individuals for whom we know the contact relations and the tuberculous history of the ancestry for two generations back.

These results are a striking illustration of the caution which must be exercised in drawing conclusions about the inheritance factor in the etiology of tuberculosis. We note:

1. Where there is no immediate tuberculous ancestry (parents and grandparents nontuberculous) 7.4 per cent of the offspring are actively tuberculous. This probably represents a little less than the normal incidence rate of the disease in the general working class population in Baltimore. Of this 7.4 per cent, however, nearly a fourth (exactly 22.2 per cent) were known to have lived for some time before developing the disease in close contact with an active case or cases of tuberculosis. On the other hand, of the 92.6 per cent of montuberculous offspring of nontuberculous ancestry only about one-tenth (exactly 11.2 per cent) had been in close contact with an active case. In other words, twice as many of the tuberculous offspring of nontuberculous ancestry had been

TABLE 4

Contact relations of individuals in family histories

ANCESTRY	OFFSPRING							
	Nontuberculous		Tuberculous		Suspect		Total	
	Close contact	No close contact	Close contact	No close contact	Close contact	No close contact	Close contact	No close contact
No parent or grandparent tuberculous....	38	300	5	21	1	0	44	321
No parent, one grandparent tuberculous..	16	65	5	10	2	0	23	75
One parent, no grandparent tuberculous..	46	13	15	1	10	0	71	14
One parent, one grandparent tuberculous.	43	19	5	1	0	0	48	20
One parent, two grandparents tuberculous.	11	4	2	0	0	0	13	4
One parent, three grandparents tuberculous..................................	2	0	0	0	0	0	2	0
Two parents, no grandparent tuberculous.	20	5	13	1	3	0	36	6
Two parents, one grandparent tuberculous.	6	3	4	0	0	0	10	3
Totals..............................	182	409	49	34	16	0	247	443

TABLE 5

Percentage of close contact in different ancestral groups

ANCESTRY	PER CENT OF TOTAL OFFSPRING TUBERCULOUS	PER CENT OF TUBERCULOUS OFFSPRING IN CLOSE CONTACT	PER CENT OF NONTUBERCULOUS OFFSPRING IN CLOSE CONTACT
No parent or grandparent tuberculous..........	7.4	22.2	11.2
No parent, one or more grandparents tuberculous	17.3	41.2	19.8
One parent, and no, one or more grandparents tuberculous..................................	19.8	94.1	73.9
Two parents, and no, or one grandparent tuberculous..................................	38.2	95.2	76.5

in close contact with active, open tuberculosis, as had been the case with the nontuberculous offspring of nontuberculous ancestry.

2. As we pass to the next ancestral rubric, which includes cases where neither parent was tuberculous, but one or more of the grandparents were, it is seen that the percentages all increase. Seventeen and three-tenths of the offspring are tuberculous, but nearly one-half of these tuberculous offspring (exactly 41.2 per cent) were for some time before acquiring the disease in close and intimate contact with an active open case or cases. In the case of the 58.8 per cent of nontuberculous offspring, having the same type of ancestry relative to tuberculosis, only about one-fifth (actually, 19.18 per cent) had been in such close contact with active open cases. Or again we see, just as in the nontuberculous ancestry rubic, that relatively about twice as many of the offspring who developed clinically active tuberculosis had been in close contact with other tuberculous cases as in the case of the non-tuberculous offspring of the same type of ancestry.

3. The next line of the table includes the cases of still more pronounced tuberculous ancestry. Here are brought together all of the offspring who had one parent tuberculous, and no, one, or more grandparents tuberculous. In this case, the percentage of the total tuberculous offspring rises only a little above that in the preceding rubric, the increase amounting to only 2.5 per cent, but the contact rates show a relatively enormous difference. Whereas, about one-fifth of the offspring of this type of ancestry themselves show active tuberculosis, nearly all of the individuals who do exhibit the disease (exactly 94.1 per cent) are known to have been in close contact with one or more clinically active cases for some time before they broke down with the disease. Of course, this enormous increase in the percentage is accounted for, to a large degree, by the fact that one parent is tuberculous, and the children have lived with this parent while he or she was actively tuberculous, but before they (the children) acquired the disease. But whatever the explanation the difficulty in interpreting the data from the standpoint of pure inheritance is obvious. An exactly similar, though not so large, increase is found when we examine the nontuberculous offspring of this same type of ancestry, where one parent, and no, one, or more grandparents are tuberculous. Of the nontuberculous offspring of such ancestry 73.9 per cent have been in close contact for some time with active open cases of tuberculosis.

4. In the last line of the table we have the cases where both parents, and no or one grandparent were tuberculous. Here we find 38.2 per cent of the offspring tuberculous. Of these tuberculous individuals, 95.2 per cent were known to have been for some time in close contact with an active open case or cases of tuberculosis before they exhibited signs of the disease. Of the 61.8 per cent nontuberculous offspring of this same ancestry, about three-fourths (exactly 76.5 per cent) had been in close contact with active open cases to just as great a degree as their tuberculous brothers and sisters without themselves developing the disease.

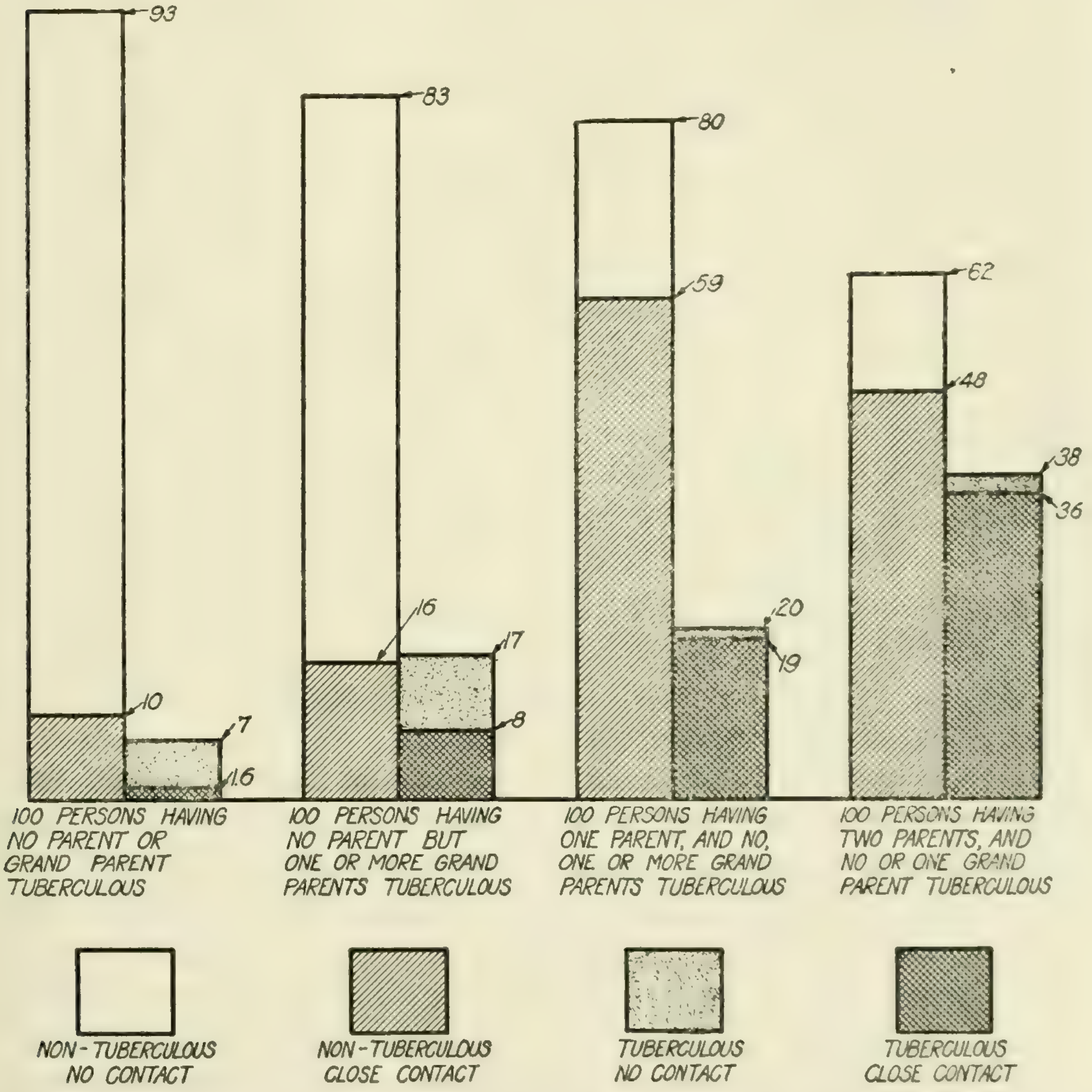

Fig. 2. Showing the Contact Relations of Tuberculous and Nontuberculous Individuals of Different Grades of Tuberculous Ancestry, Prepared from Data of Tables 4 and 5

The results of table 5 are shown graphically in figure 2.

These tables, 4 and 5, demonstrate the difficulty of interpretation which inheres in statistics regarding the inheritance of the tuberculous diathesis. As the amount of tuberculosis in the direct ancestry increases, the amount of tuberculosis in the offspring increases also, but the disturbing fact is that the rate of close contact with open active cases increases enormously more rapidly than does the rate of incidence. In short, we find that where one or both parents are actively tuberculous, practically all the offspring who subsequently develop tuberculosis have been in close, intimate contact with another active case, usually, of course, that of the parent or parents. Instantly those who oppose the view that constitution plays any part in the etiology of disease, and particularly of tuberculosis, will assert that this explains the whole matter—that if the children had not been in contact with the open, active cases, they would not have broken down with the disease. Just possibly they may be right. The case, however, is not simple. Our figures equally show that where one or both of the parents were actively tuberculous, *virtually three-fourths of the nontuberculous offspring have been in just as close contact with active open cases as their brothers and sisters who unfortunately developed the disease.* And it must not be supposed that this high contact percentage can be explained by asserting that the nontuberculous children of tables 4 and 5 are all young children who will subsequently all develop the disease. This is not true. Their average age is significantly the same as that of the tuberculous offspring.

Since the material is here used for purposes of illustration only, and since the numbers involved must be greatly increased before a final analysis can be undertaken, I shall make no attempt here to go into any further detailed analysis of this material along lines which obviously suggest themselves. The disturbing thing, and the thing which demonstrates that much further work must be done upon the problem, is that in every case the close contact rate is higher (compare the second with the third column in the table) for the tuberculous offspring than it is for the nontuberculous offspring. In other words, these figures indicate that familial contact with active open cases is beyond question a factor in determining the incidence rate of clinically active tuberculosis. It appears equally obvious, however, that it certainly does not account for the whole, and probably accounts only for a small part, of the increase in the incidence of the disease which we find to occur as the amount of tuberculosis in the immediate direct ancestry increases.

As I have repeatedly stated, these figures are to be regarded as only preliminary, and are introduced here solely for illustration of difficulties of method. Before we shall be satisfied to draw conclusions from them, or to pursue them to their ultimate possibilities in the way of mathematical analysis, we shall need to multiply the numbers many-fold. I do think, however, that the material here presented is sufficient in the first place to justify caution against accepting unreservedly any supposed measurements of the force of inheritance in determining the frequency of breakdown from tuberculosis. In the second place, I think the figures are useful as demonstrating that if the problem is ever to be solved we must obtain data of a much more detailed and accurate character than any of those which, so far as I am aware, have hitherto been used, in attempting to measure the significance of the inheritance factor.

Evidence from the natural history of the disease. We may now pass from a consideration of the direct evidence of the inheritance of the tuberculous diathesis to certain of the lines of indirect evidence, which are not less important in reaching a just conclusion regarding this important problem. A good deal of important indirect evidence may be derived from known facts regarding the natural history of the disease. Let us first in this connection examine what is known about the history of mortality from tuberculosis in old civilizations on the one hand, and newly settled countries on the other hand. Pearson was the first to point out the curious fact that the decline in the rate of mortality from tuberculosis, which in old and long settled countries had been going on at a steady rate for a good many years, ceased to proceed at the same rate almost immediately following the discovery of the bacterial causes of the disease. While to be sure the mortality rate continued to decline, this decline has proceeded at a much slower pace since the time of Koch's discovery of the bacillus, and the consequent inauguration of the active "fight against tuberculosis." Pearson first dealt in detail with the corrected mortality rates for England, and compared the phthisis death rate with the general death rate from all causes. He found during the period from 1847 to 1866, which was characterized in the case of the general death rate from all causes by a stability of course, that the death rate from phthisis fell long before the general death rate, and before what may be termed the period of sanitation. He points out that "this in itself indicates a natural rather than an artificial decay of phthisis." He further showed that during what he terms the "period of sanitation" (1866–1891) the fall in the phthisis death rate was more marked than in the general death rate. During his third or last period (1891–1910), during which time three things happened, namely, the discovery of the tubercle bacillus, the introduction of sanatoria for the treatment of tuberculosis, and the inauguration of the "fight against tuberculosis," the rate of fall in the death rate of phthisis instead of being accelerated was actually retarded.

Examination of the same sort of data from other countries in Europe, and from the part of the United States adjacent to the Atlantic seaboard, where the records run back for a considerable period of years, indicates that these phenomena which Pearson first pointed out for the tuberculosis death rate of England are apparently general and world-wide phenomena, for all biologically similar kinds of population. Owing to lack of space it is impossible to present this material here.

Recently Pearson (5) has brought his discussion of the tuberculosis death rate of England up to date. In 1911 he pointed out that the curves then available seemed to indicate that an actual rise in the phthisis death rate might in the near future be reasonably expected. The indications that this view was correct were still stronger when the returns for 1910 and 1914 were plotted. In the most recent paper he has plotted the figures to and including 1918. Owing to the difficulty in interpreting any vital statistics in a country like England during the war period it is not entirely clear whether the actually observed rise in the rate of tuberculosis mortality which the curves show is to be regarded as a real phenomenon. Pearson's very conservative conclusion in the matter is as follows:

> On the whole it is risky to form a very definite judgment, but, having regard to the female phthisis death-rate and to the percentage of the phthisis death-rate on the general death-rate, war difficulties do not seem to me sufficient to obscure the general trend of our graphs (as indicated before the war), namely that somewhere about 1915 the fall in the phthisis rate which has been less rapid since 1895 would cease altogether and probably be followed by a *rise*. The next five years will show whether this be true or not. We should expect a fall in the phthisis death-rate immediately, but on the average the value will remain higher than that of 1915.

Strong indirect evidence of the importance of the constitutional factor in the etiology of tuberculosis is afforded by the course of the tuberculosis death-rate in newly settled countries, where pioneering stock has gone to take advantage of the opportunities of an unexploited land. The course of events in all such countries where it is possible to get figures seems to be something like this. In spite of the fact that the early settlers are for the most part within the age groups where the incidence of tuberculosis is normally heaviest, namely, from say twenty to forty-five years, the tuberculosis mortality in these countries is at the outstart very low indeed, and rises, at a more or less rapid rate in different countries, the longer the country has been settled, until finally it reaches what may be regarded as a normal level for the tuberculosis death rate, having regard to the climatic conditions and the racial stocks involved, whatever they may be. As an indication of what happens in such cases figure 3 is inserted, which gives the death rate from tuberculosis for the Provinces of British Columbia and of Saskatchewan in the Dominion of Canada. For comparison over the same period of time the tuberculosis death rate of the Province of Ontario is inserted. Ontario is, of course,

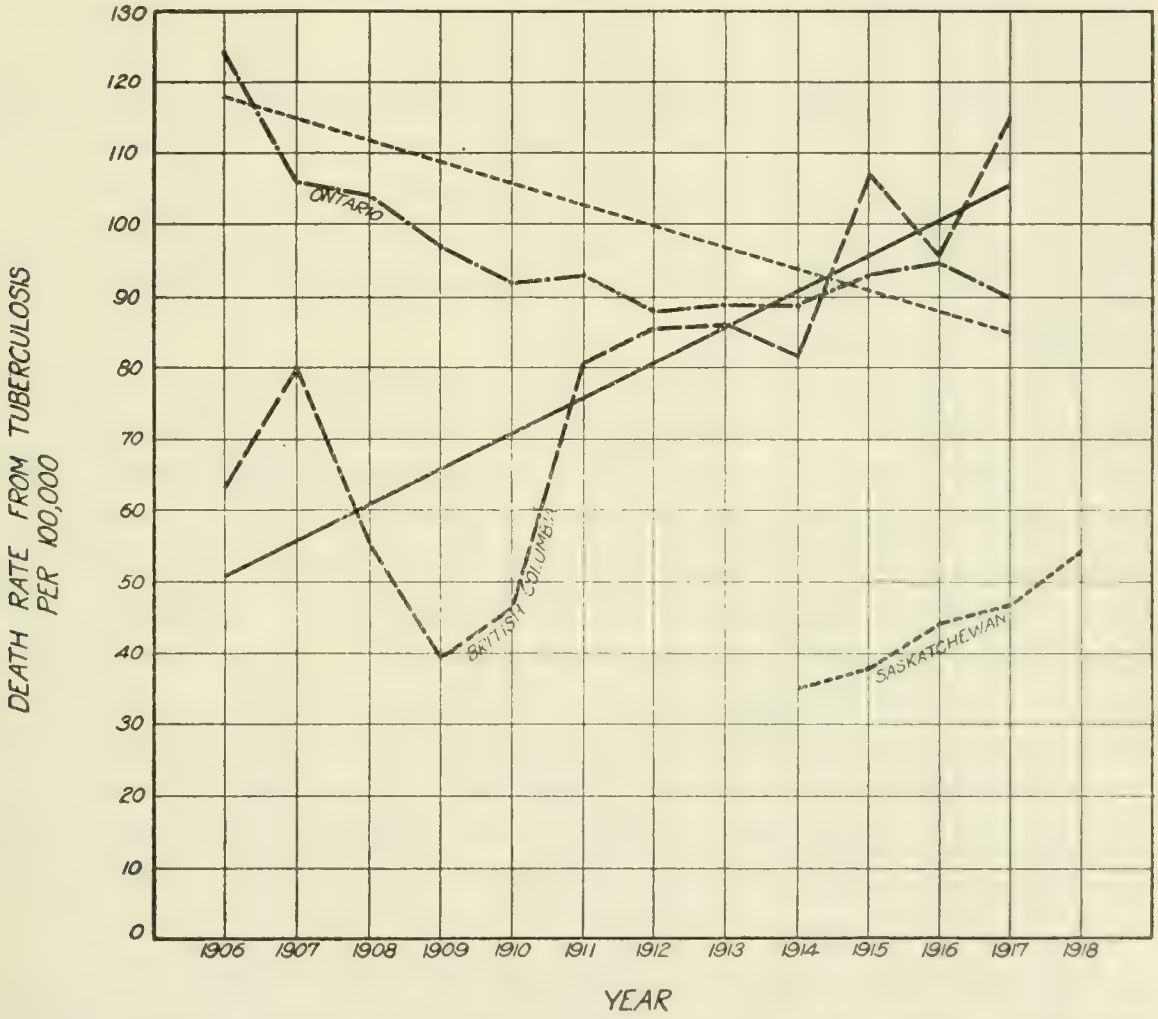

FIG. 3. DIAGRAM SHOWING THE COURSE OF THE DEATH-RATE FROM TUBERCULOSIS IN THE PROVINCES OF BRITISH COLUMBIA, SASKATCHEWAN, AND ONTARIO, CANADA

a much longer settled country than either Saskatchewan or British Columbia. Unfortunately, the number of years available for either of the new provinces is small, but the curves given will sufficiently indicate the general trend of affairs.

This diagram shows that in the relatively newly settled provinces of British Columbia and Saskatchewan the tuberculosis death rate is rising.[3] In British Columbia, which has been settled longer (it entered the Canadian Confederation in 1871) than Saskatchewan (which became a province in 1905) the rate has reached what may be regarded as a normal level and is not likely to increase much further. On the other hand the rate in Saskatchewan is extremely low *absolutely*, and is rising rapidly. In both cases the contrast with Ontario, which is inhabited by the same sort of people racially as the other two provinces mentioned, is very great. The Ontario rate is slowly falling, the normal condition in an old, long-settled country.

These results are precisely of the sort which would be expected on biological grounds. Pioneering stock—the sort of people who adventure to settle new countries—is made up mainly of constitutionally sound and vigorous people. They are of the age most liable to tuberculosis, but they are constitutionally not the kind of persons who have tuberculosis. Consequently, we find the death rate from tuberculosis in the earlier years of such settlement of new country, very low. But it steadily rises. As the new land is opened up by the pioneers and living is proved by them not to be too hazardous a business, people of weaker stocks follow them into the country. The death rate from tuberculosis in consequence begins to rise and continues steadily to do so till a stable absolute level is reached corresponding to the normal for the climatic conditions and racial stocks involved.

The phenomenon which is here illustrated by the data from these three Canadian provinces appears to be general for new countries as compared with those of long settlement.

Evidence from differential racial incidence in the same environment. It is a well known fact that different races and peoples exhibit statistically widely different rates of mortality from tuberculosis. I do not now refer to any comparison of uncivilized races initially exposed to the tubercle bacillus, with civilized races long habituated to its pres-

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[3] In order to show more clearly the trend of the mortality, straight lines have been fitted to two of the curves in fig. 3. The equation to the British Columbia curve is $y = 45.9 + 4.9x$, where y denotes death rate and x time. The Ontario curve is $y = 110.4 - 2.1x$.

TABLE 6

Comparative mortality from pulmonary tuberculosis of different race stocks in New York and Pennsylvania, 1910

SEX AND AGE PERIOD	DEATH RATES PER 100,000 LIVING AT SAME AGES, OF PERSONS BORN IN													
	Austria Hungary		Russia		Italy		Germany		England, Scotland and Wales		Ireland		United States (whites only)	
	Pennsylvania	New York	Pennsylvania	New York	Pennsylvania	New York	Pennsylvania	New York	Pennsylvania	New York	Pennsylvania	New York	Pennsylvania	New York
Males:														
All ages	118.0	166.0	107.4	114.7	81.5	112.1	194.9	267.4	150.2	215.2	342.8	589.3	105.1	170.9
Under 10	13.8	16.7	15.4	23.5	19.9	8.3	147.9			23.4			23.3	23.4
10–14	35.5	14.1		11.7	23.0	15.1		52.8		36.9			11.2	10.2
15–19	55.4	102.4	69.6	60.5	43.4	106.8		90.8	120.3	128.7	428.0	312.3	61.1	101.5
20–24	106.5	93.1	99.6	96.4	86.5	140.4	211.3	45.9	59.3	71.5	127.7	327.3	147.6	216.3
25–44	110.6	177.2	105.9	117.1	71.2	102.0	198.2	252.5	151.5	240.5	375.8	662.9	185.2	352.0
45–64	264.2	302.5	225.1	246.4	154.3	172.9	230.3	350.0	165.3	268.9	408.8	682.1	174.1	262.1
65–84	242.7	247.9	148.7	182.4	631.8	208.5	140.6	211.3	233.5	210.3	206.1	329.3	189.3	161.4
85 and over							180.2	210.7				357.6	60.0	69.3
Females:														
All ages	130.2	102.6	91.7	74.6	102.2	160.1	90.4	115.3	133.2	123.3	201.2	276.1	98.8	109.6
Under 10	27.4		31.3		42.0	34.0		51.7	41.6	23.2			19.1	20.6
10–14	17.3	13.7		5.7		89.1						138.1	19.3	27.1
15–19	74.2	49.6	70.9	28.0	102.0	220.6	39.1	76.9	84.4	64.0	63.6	185.6	91.5	111.5
20–24	141.2	87.9	92.6	88.7	153.6	247.7	128.0	158.2	80.4	121.5	121.6	167.7	162.3	186.3
25–44	152.7	137.2	108.0	104.7	114.8	159.3	106.2	125.1	166.9	165.4	235.3	353.4	172.8	193.3
45–64	162.3	122.9	124.6	80.8	77.7	123.3	85.4	109.7	112.2	92.1	220.7	250.1	108.9	111.1
65–84	215.7	142.1	178.7	94.3	217.3	55.4	72.9	106.3	153.7	139.3	140.9	192.1	178.7	137.9
85 and over						793.7	156.7	80.3	314.5	164.5	148.6		100.9	32.6

ence. It is well known that the ravages of this disease, as well as many others, are terrific among people meeting it for the first time. Such facts have little if any evidential bearing upon our present problem. There is, however, another set of facts which appears to be of considerable significance in indicating that genetic constitution plays an important part in the etiology of the disease. This is the widely different rates of fatal breakdown from tuberculosis among different racial stocks living in the same general environment. The most recent data upon the subject are furnished by Dublin and Baker (6) in a notable piece of biostatistical research.

Table 6 is compiled from their data.

From this table it is evident that different racial stocks, living under the same climatic conditions, exhibit wide differences in their mortality from tuberculosis. Contrast the Italian, with a male mortality at all ages from tuberculosis of 81.5 per 100,000 in Pennsylvania, with that of Irish males in the same state, which reaches the value of 342.8 per 100,000. For the same two race stocks the females show in the same state mortality rates of 102.2 and 201.2 per hundred thousand respectively. It is evident that the Irish react to the same environment in a totally different way than do the Italians in respect of tuberculosis. The relatively great racial susceptibility of the Irish to this disease is well-known. It appears wherever vital statistics are recorded.

There are many interesting points of detail which might be discussed in connection with table 6, but space is lacking here. I merely insert the table as a standing demonstration of the broad general fact that there are wide differences in respect of mortality from tuberculosis among different race stocks living in the same general environment. These differences are larger in amount than can reasonably be explained by any differences which may be supposed to exist, or do in fact exist, between the different stocks in respect of habits of living, social, or economic status, or other environmental factors.

CONCLUSION

In the brief space of an address it is wholly impossible to marshal more than the smallest fraction of the pertinent evidence which bears upon this problem. All that has been attempted here, and if that has been accomplished I am satisfied, is to demonstrate, in the first place, that the problem of the causation of breakdown with clinically active tuberculosis, is a problem of tremendous complexity and difficulty, towards the solution of which only the merest beginning has been made, and in the second place, that the inherited constitution of the individual is a factor in the problem of a great deal more than the negligible importance which some in high positions would accord it. I realize as clearly as my critics, potential and actual, possibly can that the evidence which I have presented here, as well as practically all of the other evidence now existing regarding the role of the constitutional factor in tuberculosis, is capable of other interpretation than the one here suggested. I personally think that these other possible interpretations of the facts I have set forth are extremely improbable, and that the one I have suggested is probably the correct one. I cannot as yet, however, *prove* the absolute correctness of my faith. But I also perceive with equal clearness that the environmentalists' case, the infectionists' case, and all the rest are on no better footing. The plain fact is that we are densely ignorant of the relative influence of the several factors which may be concerned in the etiology of tuberculosis. The task before the investigator is to devise and accomplish a real measurement of the relative importance of the hereditary and environmental factors in the tuberculosis problem.

With the closing remarks of Dr. William Bulloch, in the discussion following the reading of the notable paper by himself and Dr. Major Greenwood before the Royal Society of Medicine, I find myself in complete agreement. Doctor Bulloch said that the object in presenting the paper was:

> to enter a protest against the wild statements now being made in the lay and medical press, that the whole problem of phthisis was one of infection. Medical history showed that in tuberculosis, as also in the case of other diseases, the most extreme views were taken, not by those who had contributed the actual advancement in knowledge, but by those whose business it was to apply those advancements for the needs of the public. There were a large number of well-ascertained facts which were not entirely explicable on the doctrine that disposition was not an important factor in the genesis of the disease, and that before rigorous measures were applied on a wide scale the actual facts should be ascertained. He did not agree that public health authorities must always "do something." This "doing something" should be put a stop to until there was a reasonable supposition that it was going to achieve its end. He did not wish it to be understood that the tubercle bacillus was not a potent factor. What he did refuse to believe was that it was the only factor. He considered that the disposition, the power of the individual

to resist the agressive inroads of the bacillus, was greater than many people held at the present day.

Finally, I wish also to quote the closing words of a recent notable paper by Dr. S. Lyle Cummins (7). He says: "These considerations indicate that exhaustive research is still necessary in order that efforts at the control of tuberculosis may be directed upon effective lines." With this opinion I most heartily agree.

REFERENCES

(1) Brown, L., Petroff, S. A., and Pesquera, G.: Etiological studies in tuberculosis. Amer. Rev. Tuberc., 1919, iii, 621.

(2) Fishberg, M.: Pulmonary tuberculosis. Lea & Febiger, Philadelphia and New York, 2nd edition, 1919, p. 58.

(3) Bulloch, W., and Greenwood, M.: The problem of tuberculosis considered from the standpoint of disposition. Proc. Roy. Soc. Med, May., 1911, iv, Epidem. Sect., 147–184.

(4) Pearson, K.: Tuberculosis, heredity and environment. Eugenics Laboratory Lecture Series, 1912, viii. This is a popular summary of the work mentioned on p. 694.

(5) Pearson, K.: The check to the fall in the phthisis death-rate since the discovery of the tubercle bacillus and the adoption of modern treatment. Biometrika, 1919, xii, 374.

(6) Dublin, L. I., and Baker, G. W.: The mortality of race stocks in Pennsylvania and New York, 1910. Quart. Publ. Stat. Assoc., 1920, xvii, 13.

(7) Cummins, S. L.: Tuberculosis in primitive tribes and its bearing on the tuberculosis of civilized communities. Internat. Jour. Pub. Health, 1920, i, 137.

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A FURTHER NOTE ON WAR AND POPULATION[1]

IN a note published last summer[2] I drew atention to the course of the ratio

$$\frac{100\ \text{Deaths}}{\text{Births}}$$

n the principal belligerent countries of Europe between 1913 and 1918. All of the curves presented, with the single exception of that for Prussia, ended on a high point in 1918. The question was raised as to what would be their course after that year, and it was shown that England and Wales gave a value of 73 per cent. for 1919 against 92 per cent. for the high point n 1918. The first three quarters of the year 1920 give for England and Wales a value of 46.8 per cent. This is 10 points *lower* than the figure for 1913! For every death England had more than two births.

The *Journal Officiel* has recently published the 1919 figures for France (77 non-invaded departments only) to the following effect:

$$\frac{100\ D}{B} = \frac{63569400}{413379} = 154 \text{ per cent.}$$

This figure compares (for the same territory) with 198 in 1918, 179 in 1917, 193 in 1916, 169 n 1915, 110 in 1914, and 97 in 1913. In other words, in the next year immediately following he cessation of hostilities France's death-birth atio came back to less than that of 1915, the rst whole year of the war. With an increase f 157 per cent. in marriages in 1919 over 1918 there seems little risk in predicting that 1920 ill show a ratio not far from 100, which will be about the normal prewar status, France having had for some time a nearly stationary population. The 1920 vital index for France may well prove to be considerably below 100.

Another, and even more striking illustration of the exceedingly transitory effect of war upon the rate of population growth, is seen in the figures for the City of Vienna. Probably no large city suffered so severely from the war as did this capital. Yet observe what has happened, as set forth in Table I. To this table I have added, for the sake of rounding out the data of this and the former paper, the death-birth ratios of the United States Registration Area for as many years as they are available, and for England and Wales, 1912 to 1920 (first three quarters of latter year).

TABLE I

Percentage of Deaths to Births

Year	City of Vienna	U. S. Birth Registration Area	England and Wales
1912	80	—	56
1913	85	—	57
1914	86	—	59
1915	113	56	69
1916	143	59	65
1917	195	57	75
1918	229	73	92
1919	162	58	73
1920	—	—	47[3]

These figures are shown graphically in Figure 1.

We note that:

1. The high point of the Vienna curve in 1918, 229 per cent., is higher than that for France (198 per cent.), and probably higher than for any other equally large aggregate of population in the world.

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[1] Papers from the Department of Biometry and ital Statistics, school of hygiene and public ealth, Johns Hopkins University, No. 27.

[2] Pearl, R., SCIENCE, N. S., Vol. LI., pp. 553–4. 1920.

[3] First three quarters of year only.

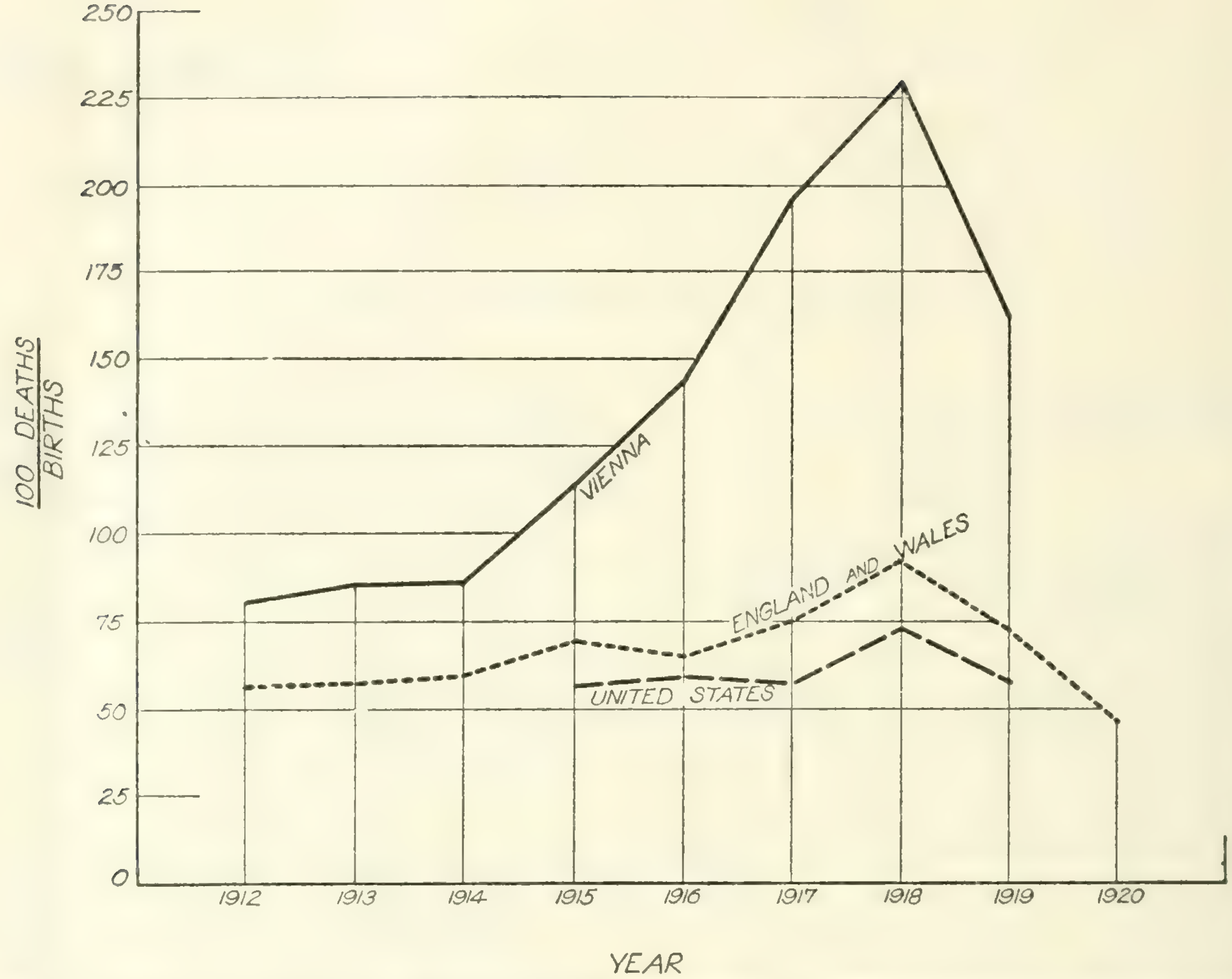

FIG. 1. Showing the change in percentage which deaths were of births in each of the years 1912 to 1919 for Vienna (———); 1915 to 1919 for the United States (— —); and 1912 to 1920 for England and Wales (-----).

2. The drop in 1919 is sharp in its angle and marked in its amount, the percentage coming down nearly to the 1916 figure—and this in spite of the very distressing conditions which prevailed in Vienna throughout 1919. It is not at all improbable, indeed rather it is probable that Vienna will in 1920 show a ratio under 100—that is, more births than deaths. If this happens she will have begun absolute natural increase again in only the second year after the cessation of hostilities, during the last year of which she had 2¼ persons die for every one born.

3. The war produced no effect upon the death-birth ratio in this country, as would have been expected. The influenza epidemic in 1918 raised the curve a little, but it promptly dropped back to normal in 1919.

4. In England and Wales the provisional figure indicates that 1920 will show a lower vital index than that country has had for many years.

Altogether, these examples, which include the effects of the most destructive war known to modern man, and the most devastating epidemic since the Middle Ages, furnish a substantial demonstration of the fact that population growth is a highly self-regulated biological phenomenon. Those persons who see in war and pestilence any absolute solution of the world problem of population, as postulated by Malthus, are optimists indeed. As a matter of fact, all history definitely tells us, and recen history fairly shouts in its emphasis, that such events make the merest ephemeral flicker in the steady onward march of population growth

RAYMOND PEARL

TREASURY DEPARTMENT
UNITED STATES PUBLIC HEALTH SERVICE
HUGH S. CUMMING, SURGEON GENERAL

INFLUENZA STUDIES

II. Further Data on the Correlation of Explosiveness of Outbreak of the 1918 Epidemic

III. On the Correlation of Destructiveness of the 1918 Epidemic

IV. On the Correlation Between Explosiveness and Total Destructiveness of the Epidemic Mortality

BY

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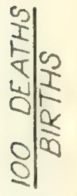

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4. In England and Wales the provisional fig-

RAYMOND PEARL

INFLUENZA STUDIES.[1]

By Raymond Pearl, Ph. D., D. Sc., LL. D., Professor of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University; Consultant in Vital Statistics and Epidemiology, United States Public Health Service.

II. FURTHER DATA ON THE CORRELATION OF EXPLOSIVENESS OF OUTBREAK OF THE 1918 EPIDEMIC.[2]

I. Introduction.

In the first of these Studies[3] it was shown that there was a definite and sensible net correlation between explosiveness of outbreak of the epidemic, as measured by an epidemicity index, and the normal death rate from certain organic and chronic diseases. Because of the importance of the subject it has been thought desirable to reexamine critically the data, making use of more refined quantitative measures of the several variables dealt with. It is the object of the present paper to give the results of this re-study of the problem. As before, the basic data are from the large American cities for which weekly data were furnished during the epidemic, by the Bureau of the Census.[4] There is now in progress in this laboratory an extended study of the same problems on the basis of data from the 96 great towns of England and Wales, as well as further studies on the American data.

Before taking up the detailed matters of the present study, I should like to call attention briefly to some methodological considerations which lie at the foundation of this and other papers in this series. It is hoped that in this way the nonmathematical reader may gain a more adequate conception of the real meaning of the results.

The most useful general method of acquiring knowledge of dynamic phenomena is unquestionably the experimental method. When we deal with phenomena of human biology, there is a wide range of matters in which the laboratory experimental method is, in the nature of the case, ruled out. Unfortunately, one can not breed homozygous strains of men at will for experimental purposes, nor subject them methodically to desired environmental conditions. In studying most problems of human biology resort must be had to some form of the statistical method. This is fundamentally a descriptive method, and hence in many of its phases is ill adapted to the analysis of dynamically active events. There is, however, one branch of the statistical calculus which offers certain methodological possibilities which I think have been generally overlooked. Let us consider this.

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[1] Reprint from the Public Health Reports, vol. 36, No. 7, Feb. 18, 1921, pp. 273-298.

[2] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 21.

[3] Pearl, R., Influenza Studies. I. On Certain General Statistical Aspects of the 1918 Epidemic in American Cities. Public Health Reports, vol. 34, No. 32, pp. 1743-1783, 1919. Reprint No. 548, pp. 1-43. All citations in this paper are in terms of the pagination of the reprint.

[4] It is a pleasure to express my indebtedness, for help with the extremely laborious computation of multiple correlation coefficients used in this work, to my assistants Mr. John Rice Miner, Mrs. [illegible] Howell, and Miss Agnes L. Bacon.

The essence of the experimental method, as practiced in the laboratory, and in theory, is that, of the multitude of variables conditioning a phenomenon, as many as possible are, by appropriate methods, held constant while one variable (or at most a very few selected variables) is allowed to vary and the results are noted. One may then deduce the relative significance of the selected variable in determining the phenomenon under observation. Now we frequently hear in scientific discussions about the experiments that Nature makes. Actually the true conditions of an experiment are rarely if ever realized in the course of natural events. It is just because Nature permits manifold and haphazard changes in *all* variables at the same time that recourse must be had to the method of experimental control in the laboratory. What is needed in order to interpret the results, in the experimental sense, and determine the meaning of the manifold and ceaseless changes and variations in the flow of naturally determined events, is some method of picking out of the manifold some selected *constant* conditions of a series of variables, and then measuring the extent and character of the variations in a *single* selected variable, whose true relative influence upon the phenomenon it is desired to know, while all these other variables are held constant. If this can be done we shall have realized all the epistemological advantages of the experimental method as practiced in the laboratory, and have freed ourselves at the same time from the limitations which in so many cases inhere in the material itself and make the laboratory type of experimental inquiry impossible. In other words, we shall have let Nature perform the experiment, in the sense of determining the phenomena, in her own way, while we evaluate the results in critically analytical terms of precisely the same sort and meaning as those in which we evaluate the results of a laboratory experiment.

Now exactly this epistemological boon is actually afforded in the method of partial or net correlation, if properly handled. This analytically simple, if geometrically complex, calculus enables one, out of a manifold complex of variables operating in an entirely uncontrolled and natural manner, to determine the variation of any selected single variable, or the correlation of any selected pair for *constant* conditions or values of the other variables in the complex. I judge that the epistemological possibilities of this method are not

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yet fully grasped by scientific men generally. When they are I believe it will rank as a fundamental method of acquiring knowledge, combining certain of the advantages of the descriptive or historical and of the experimental methods. It seems to me much more effectively to justify Royce's [5] eulogy of the statistical method than any of the arguments which he advanced. The best elementary, but at the same time adequate, account of the method is that of Yule.[6]

The problem with which this paper has to do may be stated in this way: It is an obvious fact that the large American cities varied enormously among themselves in respect to the explosiveness of outbreak of epidemic mortality in the autumn of 1918. What factors, environmental or other, were significant in determining or influencing this variation? Or, put in another way, what factors of the numerous other respects in which these 34 large cities differ from one another can be shown to be significantly correlated with the observed differences in explosiveness of outbreak of epidemic mortality?

II. Variables Discussed.

The variable phenomena or attributes discussed in the present paper are listed below, together with the subscript numbers by which they will be designated in this and subsequent papers in this series.

Subscript No.	Variable.
1.	Explosiveness of outbreak of epidemic mortality as measured by an epidemicity index I_6.
3a.	Normal death rate from pulmonary tuberculosis.
3b.	Normal death rate from organic diseases of the heart.
3c.	Normal death rate from acute nephritis and Bright's disease.
3d.	Normal death rate from typhoid fever.
3e.	Normal death rate from cancer and other malignant tumors.
3f.	Normal death rate from all causes.
4.	Age distribution of population.
5.	Sex ratio of population.
6.	Density of population.
7.	Latitude.
8.	Longitude.
9.	Rate of growth of population, 1900-1910.

In the following paragraphs these variables are defined and discussed in detail.

1. In the first of these Studies a number of indices of explosiveness of outbreak of epidemic mortality were considered and the one finally adopted, I_5, was called the peak-time ratio and defined as follows:

$$I_5 = \frac{P - M'}{T},$$

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[5] Royce, J., The Mechanical, the Historical, and the Statistical: Science, N. S. 1914.

[6] Yule, G. Udny, An Introduction to the Theory of Statistics, Fifth Edition, pp. 228-253, London, 1919.

where P denotes the maximum peak mortality rate observed during the duration, T, of the epidemic, which was defined as follows: "The epidemic mortality was considered to have begun in any city on the date when the mortality curve for that city first passed outside the range of fluctuation exhibited by the curve between the week ending July 6, 1918, and the end of the week immediately preceding the epidemic rise of the curve. The mortality of the first epidemic outbreak was considered to have ended on the date when the curve again passed within the same range of fluctuation." M' is the mean weekly annual death rate in the period from July 6, 1918, to the outbreak of the epidemic.

This index, I_5, increases as the explosiveness of the outbreak increases. After the publication of the paper, however, it was pointed out by Dr. W. H. Frost and Mr. Edgar Sydenstricker, of the United States Public Health Service, that it was open to some criticism as a measure of explosiveness of epidemic outbreak, in the strictest sense of the term. The point of criticism was that inasmuch as T included the whole time within which the mortality curve was outside the normal range, the value of the index would be influenced by both the ascending and descending limbs of the epidemic curve; whereas if it is strict explosiveness of *outbreak* that we wish to measure, only the ascending limb is of moment. Reflection shows that the point is well taken, and consequently in the present study we have used an index I_6, for which the symbolic expression is:

$$I_6 = \frac{P - M'}{T'} .$$

Here the letters have the same significance as before except that T' is the number of weeks elapsed between (a) the date when the mortality curve first passed outside the range of fluctuation exhibited by the curve between the week ended July 6, 1918, and the end of the week immediately preceding the epidemic rise of the curve, and (b) the week in which the mortality curve attained its first epidemic peak. In other words, there is now included in the epidemicity index only the ascending limb of the epidemic curve. As a matter of fact, in the American cities here dealt with, little practical difference is made in any conclusions regarding the epidemic whether one uses I_5 or I_6, but there can be no question that theoretically I_6 is the superior value, and consequently we have used it in this part and the following parts of these Studies.

2. The subscript 2 refers to total destructiveness of the epidemic, a variable not discussed in the present paper, but defined in the next following of these Studies.

3. The subscript 3 refers to a normal death rate in the community from one of the causes specified by letters. In the earlier study the

death rate of the community for a single year was taken as indicative of the normal for the purposes of that work. In the present study we have taken instead the mean annual death rate from each of the specified causes for the three years 1915, 1916, and 1917. It is beyond question that the three-year average will give a much more accurate representation of the prevailing normal rate of mortality from each of these diseases in the community just preceding the epidemic than will the rate for any single year.

4. Age distribution of the population. In the first of these Studies there was used, as a single numerical index of the age distribution, a quantity which measured the extent to which each city deviates in the age constitution of its population from a fixed standard, but did not tell the nature or kind of deviation. Since the publication of that paper there has been devised a new and more adequate index[7] of the age constitution of the population. We have adopted as an age-constitution index the function

$$\phi = S\left\{\frac{\Delta^2}{P}\right\}(M - M_p)$$

where Δ is the deviation for each of six age groups (viz, 0–4, 5–14, 15–24, 25–44, 45–64, 65 and over) of the percentage of the actual population of each city in 1910 in each age group, from the percentage in the same group in the standard population of Glover's[8] life table, denoted in the formula by P; S denotes summation of all six values; M = mean age of living population in any community; M_p = mean age of persons in a stationary population unaffected by migration and which, assuming the mortality rates of Glover's life table, would result if 100,000 persons were born alive uniformly throughout each year (M_p calculated from L_x line of Glover's table (p. 16), $= 33.796$ years).

This procedure simply multiplies our former index χ^2 by the difference (given its proper sign) between the mean age of the observed population and the mean age of the standard population on the basis of which χ^2 was calculated. Since, in fact, the mean age of any actual urban population is never likely to be as great as the mean age of the stationary population chosen as a standard of reference, the actual values of ϕ will practically always be negative for cities. The smaller these negative values are numerically, the greater will be the proportion of older persons in the population concerned. In short, this function ϕ tells us not only the degree to which a given population deviates in its age distribution from a fixed standard age distribution, but also the nature of this deviation, whether on the one hand in the direction of a relative excess of aged, or on the other hand in the direction of a relative excess of the young. Theoretically it is possible for two populations differing from one another in a compensatory way to give the same values for the index ϕ. But two populations which differ in age distribution in any fundamental respect which could affect appreciably crude death rates will, in all populations I have been able to test, give different values of ϕ, provided the age classification from which the function is calculated is finely enough divided.

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[7] Cf. Pearl, R., On a Single Numerical Index of the Age Distribution of a Population: Proc. Nat. Acad. Sci., vol. 6, pp. 427-431, 1920.

[8] Glover, J. W., United States Life Tables, 1910, Bureau of the Census, 1916.

5. As an index of the sex distribution of the population, the male sex ratio was expressed as the ratio of males to 100 females in 1910.

6. The density of population in each city was calculated from data furnished in the "Financial Statistics of Cities," issued annually by the Bureau of the Census, and was expressed as the number of persons per acre of land area within the legally defined limits of the city.

7 and 8. In the earlier study the geographical position of the cities was indicated by the linear distance of each from Boston, as measured on the map. This was recognized as a very rough approximation. The interest in having some measure, in a study of this kind, of geographical position is twofold: First, that which arises from purely epidemiological considerations, namely, as affording, in relation to time, an index of the rate of spread of an infectious epidemic disease from a primary focus; and secondly, the fact that geographical position, especially latitude, is a rough but on the whole fairly accurate index of general climatological conditions. It was decided to make the expression of geographical position more accurate in the present study, and consequently there were included as definite variables the latitude and longitude of each of the cities considered.

9. Rate of growth of population 1900–1910. The reason for the inclusion of this variable in the study was twofold. Primarily this may be taken as a rough but probably fairly accurate index of the degree of industrialization of a city. In general, those cities which are growing most rapidly in population are those in which the most rapid industrial development is taking place. It would be much better if the rate of growth of the population between 1910 and the outbreak of the epidemic could have been used; but accurate data are lacking, nor will they be available until the results of the 1920 census are published. Consequently this variable must be regarded as a rough approximation to one that we should like to measure more accurately, namely, the present state and recent rate of industrial development. In the second place, rate of growth is a definite biological characteristic of a population,[9] and as such

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[9] Cf. Pearl, R., and Reed, L. J., On the Rate of Growth of the Population of the United States since 1790 and its Mathematical Representation. Proc. Nat. Acad. Sci., Vol. 6, pp. 275–288, 1920.

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worthy of inclusion in any study relating an epidemic disease to demographic conditions.

III. Data.

The actual data used in the correlations are given in Table 1. Since in other work it was desired to correlate destructiveness of the epidemic, as measured by the 25-week excess mortality, with the other variables, only 34 cities could be used, because only for that number are the excess mortality figures available.

TABLE 1.—*Data for correlation of characteristics of cities with explosiveness of epidemic influenza mortality.*

(The subscript numbers at the heads of columns correspond to the list of variables given on p. 5.)

City.	1.	3a.	3b.	3c.	3d.	3e.	3f.	4.	5.	6.	7.	8.	9.
Albany, N. Y	32.2	224.5	236.4	180.1	10.8	137.6	1,947.0	−10.73	92.9	8.89	42.65°	73.75°	6.5
Atlanta, Ga	5.3	113.9	121.6	159.2	19.5	66.5	1,551.2	−82.71	92.7	11.42	33.73	84.33	72.3
Baltimore, Md	43.4	202.7	195.9	179.0	18.1	107.5	1,810.3	−31.80	92.1	30.57	39.28	76.62	9.7
Boston, Mass	28.8	143.8	211.5	101.0	3.9	117.0	1,651.6	−31.05	96.7	27.36	42.36	71.06	19.6
Buffalo, N. Y	21.1	146.0	165.9	126.0	10.3	97.8	1,565.4	−48.61	100.6	18.97	42.88	78.92	20.2
Cambridge, Mass	17.9	178.8	183.9	73.6	2.7	118.8	1,354.4	−30.26	91.7	28.23	42.38	71.13	14.1
Chicago Ill	13.2	139.5	159.1	110.4	4.1	88.8	1,457.1	−68.76	106.3	20.28	41.88	87.60	28.7
Cincinnati, Ohio	9.2	206.8	202.5	162.0	4.8	111.5	1,618.0	−22.93	95.4	9.10	38.14	84.42	11.6
Cleveland, Ohio	17.7	132.8	118.8	90.0	7.0	82.4	1,462.1	−74.51	106.6	20.08	41.50	81.70	46.9
Columbus, Ohio	11.0	131.8	155.2	88.0	11.4	105.1	1,498.3	−29.08	101.5	15.18	40.00	83.00	44.6
Dayton, Ohio	28.8	139.2	184.3	106.2	17.3	107.7	1,492.7	−24.10	101.9	12.65	39.73	84.18	36.6
Fall River, Mass	27.8	139.6	164.0	108.5	14.3	83.9	1,639.4	−73.39	93.4	5.91	41.70	71.15	13.8
Grand Rapids, Mich	2.5	76.3	146.4	94.7	19.0	98.4	1,258.8	−27.95	97.4	11.85	42.97	85.70	28.6
Indianapolis, Ind	8.6	164.2	185.7	111.1	17.1	95.3	1,550.2	−24.23	98.7	10.93	39.67	86.13	38.1
Louisville, Ky	21.5	160.1	161.7	160.1	14.2	83.5	1,542.1	−32.19	94.1	16.61	38.20	85.70	9.4
Los Angeles, Calif	10.0	182.1	156.0	106.8	4.7	103.5	1,238.2	−13.61	103.9	2.40	34.08	118.20	211.5
Lowell, Mass	24.7	112.5	157.7	90.7	11.2	79.8	1,681.9	−34.91	94.1	13.63	42.65	71.32	11.9
Milwaukee, Wis	4.9	82.4	100.7	76.7	8.8	85.3	1,214.4	−62.17	102.8	26.92	43.05	87.95	31.0
Minneapolis, Minn	4.1	120.4	114.1	101.3	6.5	93.8	1,189.4	−55.39	109.2	11.27	44.97	93.30	48.7
Nashville, Tenn	41.5	189.3	198.7	126.1	30.0	83.3	1,683.7	−48.32	89.6	10.11	36.15	86.80	36.5
Newark, N. J	14.1	151.0	148.3	137.1	4.3	88.5	1,412.2	−62.54	99.6	27.52	40.75	74.17	41.2
New Haven, Conn	11.1	103.6	185.4	125.1	13.0	112.1	1,660.0	−33.70	99.7	13.06	41.32	72.92	23.7
New Orleans, La	34.1	270.9	224.4	245.6	22.3	95.5	1,983.0	−52.44	92.8	2.95	29.97	90.08	18.1
New York, N. Y	12.8	160.5	165.3	128.3	4.6	85.0	1,384.7	−74.42	99.9	29.54	40.71	74.00	38.7
Oakland, Calif	15.1	98.7	187.6	87.7	4.4	94.2	1,082.8	−17.18	108.7	6.41	37.75	122.33	124.3
Philadelphia, Pa	47.9	169.9	203.5	178.3	6.9	97.6	1,630.1	−34.27	95.4	21.02	39.95	75.17	19.7
Pittsburgh, Pa	21.3	115.9	134.5	94.3	10.4	90.7	1,694.0	−71.77	105.1	22.81	40.43	80.03	18.2
Providence, R. I	14.0	133.3	152.8	130.2	6.4	99.1	1,527.0	−30.05	95.7	22.35	41.84	71.40	27.8
Rochester, N. Y	13.9	95.7	203.1	138.0	4.7	109.8	1,456.8	−26.62	98.7	18.62	43.13	77.85	34.2
St. Louis, Mo	5.4	132.2	141.3	171.9	8.1	97.3	1,458.0	−44.57	101.5	19.36	38.63	90.20	19.4
St. Paul, Minn	4.5	105.6	118.9	79.7	5.0	86.2	1,093.0	−68.95	108.6	7.40	44.87	93.08	31.7
San Francisco, Calif	25.4	170.0	237.2	132.5	5.7	138.0	1,546.5	−34.34	131.6	17.55	37.79	122.43	21.6
Toledo, Ohio	17.9	173.7	185.2	88.7	18.0	103.9	1,705.1	−33.21	101.1	10.91	41.77	83.55	27.8
Washington, D. C	30.7	185.5	235.1	176.2	12.7	110.5	1,795.6	−20.67	91.3	9.55	38.89	77.03	18.8

IV. Demographic and Environmental Correlation.

We come now to the consideration of the results. The net influence of the several demographic and environmental factors upon explosiveness of epidemic outbreak may be first considered. As we are obviously interested in getting at the *net* influence of each factor, such as age distribution of the population, upon variation in the epidemicity index, while all the other factors for which we have data are held to constant values, we may pass at once to the fifth order correlation coefficients, without stopping for detailed consideration of the lower order coefficients leading to the final values. Such points as do need discussion in connection with these lower order coefficients will be brought out in connection with the fifth order results. The net correlations between epidemicity index (subscript 1) and each of the six demographic and environmental factors taken one at a time, with the other five held constant, are exhibited in Table 2. It is to be understood in this, and all subsequent papers in this series, that subscript numbers to the right of the decimal point denote variables held constant, and subscript numbers to the left of the point denote the variables correlated. r is, of course, simply the conventional symbol of a correlation coefficient.

TABLE II.—*Net correlation of explosiveness of outbreak (I_6) with various demographic and environmental factors.*

Variable correlated with explosiveness (I_6).	r subscripts.	Coefficient.
Age distribution of population	14.56789	+0.281 ±0.107
Sex ratio of population	15.46789	−0.001 ±0.116
Density of population	16.45789	+0.099 ±0.115
Latitude of city	17.45689	−0.369 ±0.100
Longitude of city	18.45679	−0.085 ±0.115
Rate of growth of population, 1900–1910	19.45678	−0.288 ±0.106

Taking the several variables in order we note:

1. Explosiveness of outbreak of epidemic mortality can not be positively asserted to be significantly correlated with the age distribution of the population. The coefficient $+0.281$ is less than 3 times its probable error. The plus sign means, having regard to the method of calculating age indices explained above, that so far as there is any correlation, high values of the explosiveness index I_6 tended to be associated with populations having a higher proportion of *older* persons than the average. No one of the lower order correlations of explosiveness and age index had a value as much as 3 times its probable error. The highest coefficient in the series was the fourth order $r_{14\cdot 6789} = +0.300 \pm 0.105$. The zero order $r_{14} = +0.194 \pm 0.111$. All r_{14} correlations, whatever the secondary subscripts, within the group now under consideration, are positive. In the first of these studies (*loc. cit.*, p. 38) the explosiveness-age correlation of zero order, with the less exact age distribution index, was $r_{14} = -0.262 \pm 0.101$, again a value nearly but not quite certainly significant. With border line values such as these, one can only say that in cities constant in respect to sex ratio of population, density of population, position, and rate of recent growth of population, the age composition of the population may have a slight influence in determining explosiveness of outbreak of epidemic mortality, but at best the influence must be very small.

2. The sex ratio of the population is plainly not significantly correlated with epidemicity index. The fifth order coefficient has a value -0.001 ± 0.116, which is sensibly zero. This is an interesting example of how partial or net correlations may differ from total correla-

tions. The zero order coefficient between explosiveness (I_e) and sex ratio is $r_{15} = -0.307 \pm 0.105$. This is a probably significant value, but arises not from any direct relation of variables 1 and 5, but indirectly through the relation of both of these to the positional variables 7 and 8 (latitude and longitude). In the group of cities here dealt with there is a relatively high correlation between male sex ratio and longitude, and between sex ratio and latitude, when longitude is constant. These relations are shown in the following coefficients:

$r_{57} = +0.134 \pm 0.114$ (sex ratio and latitude).
$r_{58} = +0.678 \pm 0.062$ (sex ratio and longitude).
$r_{57\cdot 8} = +0.607 \pm 0.073$ (sex ratio and latitude—longitude constant).
$r_{58\cdot 7} = +0.808 \pm 0.040$ (sex ratio and longitude—latitude constant).

Because of the inverse and nearly equal correlations of epidemicity index with latitude and longitude, the sex-ratio correlation with epidemicity index is neutralized as soon as these other variables are brought into the system. Thus while we have $r_{15} = -0.307 \pm 0.105$, we get $r_{15\cdot 78} = +0.023 \pm 0.116$, or practically zero. We may then safely conclude that the proportion of males (or of females) in the population of a city had no sensible direct influence in determining the explosiveness of outbreak of epidemic mortality.

3. The net fifth order correlation of explosiveness of outbreak of the epidemic mortality with density of population is again sensibly zero. This is true whatever the variables held constant within the group here discussed. The zero order coefficient is $r_{16} = +0.073 \pm 0.115$. Nowhere in the series does a coefficient having primary subscripts 16 rise to a value even approaching 3 times the probable error. This result, that density of population, which is the measure of urban crowding in this case, had nothing to do with determining the explosiveness of outbreak of the epidemic mortality, while surprising on grounds of purely *a priori* logic—always, by the way, most unsafe grounds—fully confirms with more critical data the result attained in the earlier study (*loc. cit.*, p. 36).

4. In the case of the correlation of explosiveness of outbreak of epidemic mortality with the latitude of the city, a very different result appears. The fifth order coefficient is $r_{17\cdot 4568} = -0.369 \pm 0.100$. This is more than three times its probable error and is probably *statistically* significant; but before drawing any conclusions about its epidemiological significance we must critically look into its genesis. In the first place it must be noted that the 34 cities with which we are dealing are not scattered at random over the United States. All but three of them are either on or east of the Mississippi River. This distribution is well shown in the map exhibited as Figure 1.

Not only are the cities mostly in the eastern half of the country, but, what is more important, they nearly all fall in a fairly narrow northeast-southwest belt. How clearly this is so is shown by the

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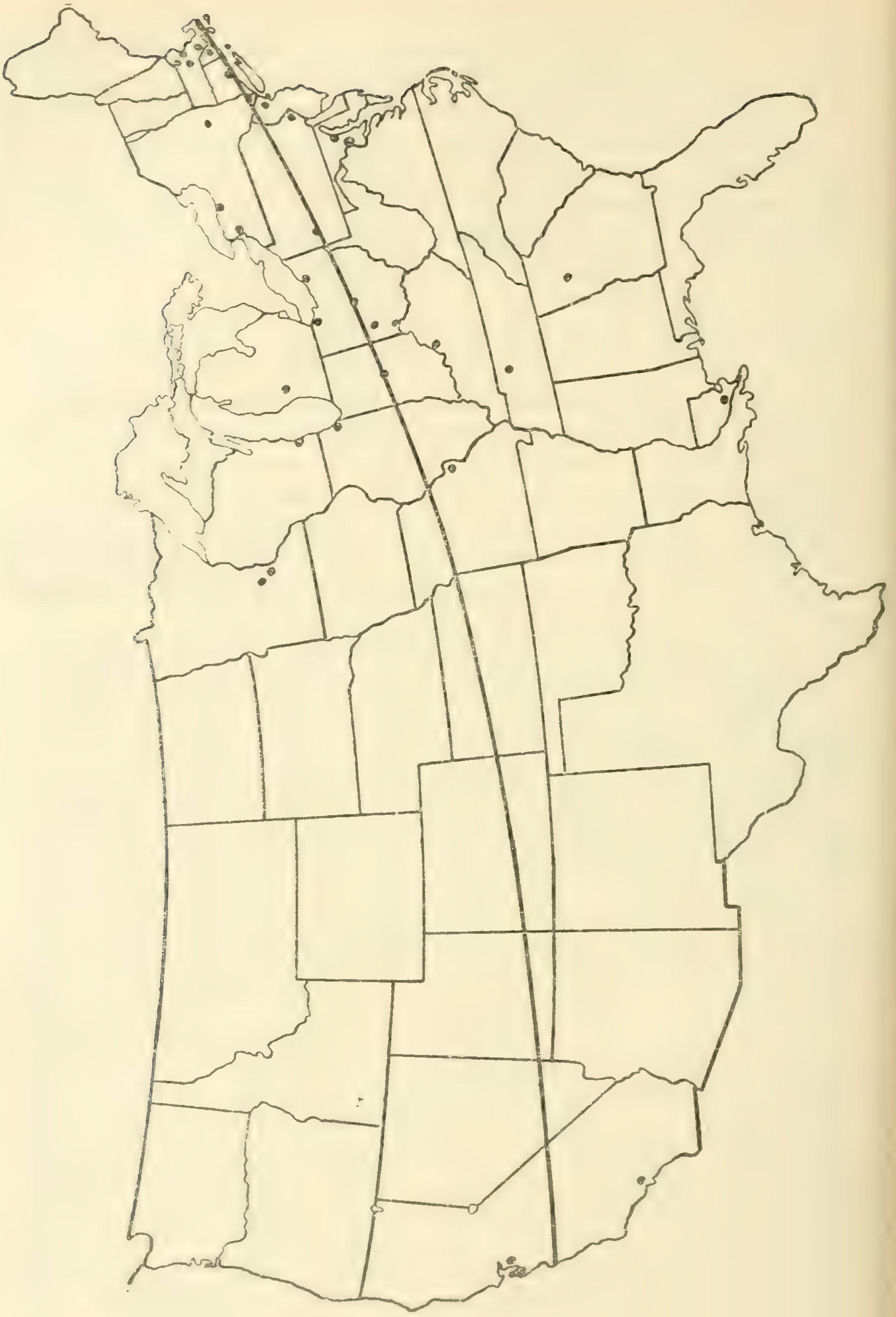

the location of the 31 cities used in this study.

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regression of latitude on longitude, which is plotted on the map.[10] The distribution of these cities is so far from random over the whole area of the country that there is a statistically significant correlation for these 34 cities, between latitude and longitude, the coefficient being $r_{78} = -0.404 \pm 0.097$. In words, what this means is that, within this group of 34 cities, in general, the farther north a city is the farther east it is.

Now, neither latitude nor longitude is alone significantly correlated with the epidemicity index I_6, as witness the zero order coefficients

$$r_{17} = -0.243 \pm 0.109$$
$$r_{18} = -0.229 \pm 0.110$$

But because of the relatively large value of r_{78} which has just been pointed out we get at once

$$r_{17.8} = -0.376 \pm 0.099 \text{ (significant)}$$
$$r_{18.7} = -0.369 \pm 0.100 \text{ (significant).}$$

The same influence makes itself felt throughout the series of ascending order partial coefficients. Thus, the relevant second order coefficients are:

$$\begin{aligned}
r_{17.45} &= -0.208 \pm 0.111 \\
r_{17.46} &= -0.303 \pm 0.105 \\
r_{17.48} &= -0.387 \pm 0.098 \\
r_{17.49} &= -0.407 \pm 0.097 \\
r_{17.56} &= -0.264 \pm 0.108 \\
r_{17.58} &= -0.319 \pm 0.104 \\
r_{17.59} &= -0.358 \pm 0.101 \\
r_{17.68} &= -0.381 \pm 0.099 \\
r_{17.69} &= -0.403 \pm 0.097 \\
r_{17.89} &= -0.421 \pm 0.095
\end{aligned}$$

$$\begin{aligned}
r_{18.45} &= -0.092 \pm 0.115 \\
r_{18.46} &= -0.242 \pm 0.109 \\
r_{18.47} &= -0.407 \pm 0.097 \\
r_{18.49} &= -0.074 \pm 0.115 \\
r_{18.56} &= +0.022 \pm 0.116 \\
r_{18.57} &= -0.245 \pm 0.109 \\
r_{18.59} &= +0.194 \pm 0.111 \\
r_{18.67} &= -0.333 \pm 0.103 \\
r_{18.69} &= -0.061 \pm 0.115 \\
r_{18.79} &= -0.160 \pm 0.113
\end{aligned}$$

It is at once evident that most of the r_{17} or latitude coefficients are three or more time their probable errors. Most of the r_{18}, or longitude coefficients are, on the contrary, less than three times their probable errors, the only ones arising to a higher value being those carrying 7 as a secondary subscript and not all of those. In other words, we come here to a separating point between the correlations carrying 1 and 7 as primary subscripts and those carrying 1 and 8 in the same position. This divergence comes about from the different correlations of certain of the other variables with latitude and longitude. Thus we have, for sex-ratio correlations, $r_{75} = +0.134 \pm 0.111$ against $r_{85} = +0.678 \pm 0.062$. Age distribution index is not significantly correlated with either latitude or longitude in this group of cities. Density is significantly and about equally correlated with both, the coefficients being $r_{86} = -0.424 \pm 0.095$, and $r_{76} = -0.371 \pm 0.100$. Rate of growth in this group of cities is nearly twice as highly correlated with longitude as with latitude, the signs of course being opposite. The coefficients are $r_{73} = -0.365 \pm 0.100$ and $r_{89} = +0.642 \pm 0.068$.

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[10] It should be noted that this regression is linear. The curvature of the regression line in Fig. 1 is merely a recognition of the fact that the projection of the map itself is such as to show latitude lines curved.

Without pursuing this complex trail further, certain things are clear. In the first place it is evident that it will be quite unsafe to draw biostatistical or demographic conclusions about, or on the basis of data from, the large American cities, without having critical regard for the fact here demonstrated that some of the most important of these characteristics are significantly correlated with the mere geographical position of the cities. In the second place, because of this fact, we can not be quite sure of the epidemiological significance of the fact that, in this group of cities, there is a statistically significant negative correlation between epidemiological index and latitude. Taken at its face value this coefficient means that, on the average, the outbreak of epidemic mortality was *more* suddenly explosive, the farther *south* the city, when the other factors of age distribution, sex ratio, density, and rate of growth of population were constant and equal. Whether this bespeaks a real and general biological phenomenon resting presumably upon a climatological base can not be critically determined until we can study the matter in a group of localities distributed in a more random manner than in the present sample, so that the correlations between latitude and longitude shall be more nearly zero in value. It is interesting to note, however, that the present result in respect to latitude correlation is in accord with general clinical and pathological experience. I am told by Dr. William H. Welch that it has long been recognized by clinicians and pathologists that acute respiratory infections, and particularly the pneumonias, tend to become more fatal as one passes from north to south. There is a splendid chance here for a critical statistical investigation of the matter, and with the rapid extension of the registration area into the South in recent years, adequate data will shortly be available.

5. Rate of growth of population of these cities in the decade 1900 to 1910 is a factor connected with age of city, with its industrial and commercial prosperity and activity, and indirectly with sanitation, because usually in cities growing very rapidly, sanitary arrangements tend to lag behind the need for them. The net fifth order correlation of this variable with explosiveness of outbreak of epidemic mortality is $r_{19.}$[illegible] $= -0.288 \pm 0.106$. This is a border-line value, which can not be safely asserted to differ significantly from zero. Roughly speaking, a value as large as this would turn up purely by chance about 7 times in every 100 trials with samples of the size here dealt with. The gross, zero order coefficient between these variables is $r_{19} = -0.302 \pm 0.105$, again not a significant value. No one of the partial coefficients having 1 and 9 as primary sub-

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scripts is certainly significant in comparison with its probable error, except such as carry also 7 as a secondary subscript. On the whole, it can be safely asserted that if the rate at which a city had recently been growing in population had anything at all to do with the degree of explosiveness of outbreak of the 1918 epidemic mortality, this influence must have been at the most extremely slight.

6. In general, the results of this more extended and critical study of the influence of these demographic and environmental factors confirm the earlier preliminary examination of the data. The only variable in the lot that has a statistically significant net correlation with the explosiveness index is latitude. In the former study, use was made of linear distance from Boston as a locality index instead of the more general and exact latitude and longitude plan here adopted. But the correlation result obtained with the cruder variables was the same in sense, and of about the same order of magnitude as the more refined net fifth order latitude correlations. I have already indicated fully the wisdom of caution for the present in drawing biological conclusions from this latitude correlation. It is possible that the age distribution of the population and the rate of recent growth of the city had some slight influence upon the explosiveness of outbreak of the epidemic mortality; but in either case the effect must have been so slight as to be negligible so far as any practical epidemiological significance is concerned.

V. Death Rate Correlations.

We may turn now to another series of correlations. In the first study it was shown that the most significant correlations of explosiveness of outbreak of epidemic mortality were with the normal death rates from certain primarily organic diseases. These results, because of their novelty and possibly far-reaching theoretical significance, have been thought particularly to need critical reexamination. As has already been pointed out, we have in the present study taken the mean death rate for three years as indicative of normal conditions instead of a single year as in the earlier work.

The sixth order coefficients are exhibited in Table III. These coefficients measure the net correlation existing between the epidemicity index I_6 and the specified normal death rate, when the cities involved have been made constant and equal in respect to age and sex constitution of population, to density and rate of recent growth of population, and to latitude and longitude.

TABLE III.—*Net correlation of explosiveness of outbreak (I_6) with the normal death rates from certain specified causes.*

Variable correlated with explosiveness (I_6). Death rate from—	r subscripts.	Coefficient.
All causes	13f. 456789	+0.572 ±0.078
Pulmonary tuberculosis	13a. 456789	+0.389 ±0.098
Organic diseases of the heart	13b. 456789	+0.562 ±0.079
Acute nephritis and Bright's disease	13c. 456789	+0.307 ±0.105
Typhoid fever	13d. 456789	+0.105 ±0.114
Cancer and other malignant tumors	13e. 456789	+0.111 ±0.113

It is at once evident that these correlations are of a generally different order of magnitude from those of Table II. Specifically we note:

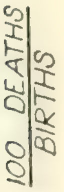
1. The highest correlation is that for the death rate from all causes; but that for organic diseases of the heart is practically identical. These coefficients are more than seven times the probable error and are certainly significant. The corresponding zero order coefficients are:

$$\text{All causes,} \quad r_{13f} = +0.678 \pm 0.063$$
$$\text{Organic heart,} \; r_{13b} = +0.642 \pm 0.068$$

From these values, in comparison with those of Table III, it appears that by making all six demographic and environmental factors constant, the correlation between explosiveness and the normal death rate from all causes or that from organic diseases of the heart, is not altered to a degree statistically significant. This comparison indicates the overwhelming importance of the biological factor which these death rates measure in determining the explosiveness of the outbreak of epidemic mortality, as compared with the demographic and environmental factors previously considered.

2. Next to organic diseases of the heart, pulmonary tuberculosis is the single cause having its normal death rate most highly correlated with explosiveness of outbreak of the epidemic. The net sixth order coefficient for this disease, however, is distinctly lower than that for organic diseases of the heart. Furthermore it is much more reduced by the process of making demographic and environmental factors constant, as is indicated by the fact that the zero order coefficient for this disease is $r_{13a} = +0.578 \pm 0.077$. The difference between this and the sixth order coefficient $r_{13a.456789} = +0.389 \pm 0.098$ is 0.189 ± 0.125. While not statistically significant in comparison with the probable error, it comes much nearer being so than the corresponding difference for the organic heart correlation, which is 0.080 ± 0.104.

3. In the case of the normal death rate from breakdown of the kidneys (acute nephritis and Bright's disease) the sixth order net coefficient is on the border line of probable statistical significance,



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having a value just under three times its probable error. Again the process of making the six demographic and environmental factors constant has materially reduced the correlation between the normal death rate from these diseases of the kidneys and the explosiveness of outbreak of epidemic mortality. The zero order correlation here is $r_{13c} = +0.447 \pm 0.093$. The difference between the zero order and the sixth order coefficients is 0.140.

4. Turning to diseases of wholly different etiology from those dealt with up to this point, namely, typhoid fever and cancer, the correlations between the normal death rate from these diseases and the explosiveness of outbreak of epidemic mortality are found to be of an entirely different order of magnitude. In neither case, typhoid or cancer, is the coefficient sensibly different from zero, having regard to its probable error. Clearly, whatever factors, biological or environmental, or both, are measured by these death rates can not have had any sensible influence in determining the suddenness with which the mortality curve rose during the influenza epidemic. It is generally held on good grounds that the typhoid death rate is an excellent index of the general sanitary status of a community. If it may be so accepted in the present connection, the result just stated bears out in precise mathematical terms what was obvious to the thoughtful and candid observer at the time of the epidemic, namely, that the severity with which a city was hit by the epidemic bore no relation to its general sanitary status or to the efficiency of its health organization. In this connection there is a further interesting mathematical point regarding the typhoid correlation. The zero order gross correlation for this death rate with I_ϵ is $r_{13d} = +0.342 \pm 0.102$. This is a statistically significant correlation; but observe that as we make the cities constant in respect to demographic and environmental factors the reduction in the correlation is very great, ending with the sixth order coefficient at a value $r_{13d.456789} = +0.105 \pm 0.114$, a drop of 0.237 in the coefficient. But this is exactly what would be expected when it is recalled to what an extent the typhoid death rate of a community depends upon the environmental conditions in that community.

5. The normal cancer death rate is not significantly correlated with the epidemicity at any stage, nor is the correlation altered to any extent by making the demographic and environmental factors constant. This is indicated by the fact that the zero order coefficient is $r_{13e} = +0.235 \pm 0.109$, while the sixth order coefficient is lower by only 0.094.

6. Taking all the results together they confirm, in general, but refine, those of the earlier study. It is now seen that a considerable part of the high correlation then found for the tuberculosis death rate with epidemicity index disappears if the cities are made constant and

equal in respect to some six important demographic and environmental factors. The correlation is still, however, in spite of the reduction, significant in comparison with its probable error. Death rate from all causes is highly correlated under all circumstances with explosiveness of outbreak. This death rate may be taken as an index of the general healthfulness of the community.[11] But the outstanding *single* factor, which apparently more than any other one thing yet discovered, determined how abruptly or explosively the mortality was to rise at the outbreak of the epidemic, was the normal death rate in the community from organic diseases of the heart. This extraordinary and striking fact will be more fully discussed in the next paper in this series.

VI. Summary.

In this second study a further more refined and detailed analysis is made of the weekly mortality statistics of the influenza epidemic of the autumn of 1918. Using the method of multiple or partial correlations the attempt is made to determine more critically the factors chiefly responsible for the great variation exhibited among 34 large American cities in respect to the degree of suddenness or explosiveness of outbreak of epidemic mortality. A new and more critically exact epidemicity index is described, as well as a more accurate single numerical index of the age distribution of a population. Every effort was made to get critical quantitative measures of the variables dealt with. In general, this more extended and refined study confirms the results of the first. It is believed that the critical refinements introduced in this and the succeeding studies fully meet the implied criticisms of Winslow and Rogers [12] on the first study, at least so far as concerns readers capable of understanding the implications of the theory of probability. It is, for example, simply idle to assert that a surely significant correlation between death rate from organic diseases of the heart and explosiveness of outbreak of influenza may be "due to peculiarities in age distribution of the population" when it is conclusively shown, as it here is, that the same correlation prevails *when the age distributions of all the populations concerned are held constant.* If the results of these studies are to be successfully controverted it must be upon a different basis than logic of this sort. Of the six environmental and demographic variables tested, only one, namely, the city's latitude, was found to have a statistically significant net correlation with the explosiveness or epidemicity index. In that case the biological or epidemiological significance of the result is doubtful, because of the peculiar nonrandom spatial distribution of the 34 cities furnishing data. On the other hand, the normal death rate from all causes and from organic diseases of the heart are correlated to a relatively high degree with explosiveness of outbreak of epidemic mortality. The normal death rate from pulmonary tuberculosis is also sensibly, but not highly, correlated with explosiveness of influenza outbreak. The normal death rates from typhoid fever and from cancer show no sensible net correlation with explosiveness.

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[11] It is so used, for example, by Brownlee, J., An Investigation into the Epidemiology of Phthisis in Great Britain and Ireland: Med. Res. Comm. Spec. Rept. Ser. No. 46, pp. 1-48, 1920.

[12] Winslow, C. E. A. and Rogers, J. F., Statistics of the 1918 Epidemic of Influenza in Connecticut: Jour. Inf. Dis., vol. 26, pp. 184-216, 1920.

III. ON THE CORRELATION OF DESTRUCTIVENESS OF THE 1918 EPIDEMIC[13]

I. Introduction.

It was pointed out by friendly critics after the appearance of the first of these Studies[14] that the epidemiological character there dealt with was explosiveness of outbreak, as measured by an epidemicity index I_5 subsequently modified into I_6 as described in the second of these Studies,[15] and that as explosiveness of outbreak of epidemic mortality was obviously a characteristic which might conceivably be nearly or quite distinct and independent from destructiveness as measured by the total number of persons killed by the epidemic, it was a matter of doubt what might be the real meaning of the biological conclusions flowing from the peculiar facts brought out in that first study, since confirmed and extended in the second. This criticism was well taken. The obvious answer to it, however, is to study destructiveness in the same way that we have explosiveness of outbreak, by the method of multiple correlation. It is the purpose of this paper to present the results of just such a study, in which the extended and critically defined series of variables of Influenza Studies II were used.

The problem to which this paper is addressed may then be stated in the following terms: The 34 large American cities for which we have available published weekly data, varied enormously among themselves in respect to the destructiveness of the epidemic, as measured by the number of their inhabitants who died in excess of the normal, during the autumn and winter of 1918–19. What factor significantly influenced or determined this variation?

The methods by which this problem is attacked in the present paper are precisely the same as those of the second of these Studies, and the reader is referred to the introductory portion of that paper for the general discussion of methods used.

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[13] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University. No. 22.

[14] Pearl, R., Influenza Studies. I. On Certain General Statistical Aspects of the 1918 Epidemic in American Cities: Public Health Reports, vol. 34, No. 32, pp. 1743-1783. 1919. Reprint No. 548, pp. 1-43. All citations in this paper are in terms of the pagination of the reprint.

[15] See p. 6.

II. Variables Discussed.

The variables discussed in the present paper are precisely the same as those listed in Influenza Studies II (p. 5), with two exceptions. These are (a) that here the variable I_6 (explosiveness of outbreak) is omitted, and (b) its place is taken by a new variable, destructiveness, which is indicated by the subscript 2 in all that follows. Destructiveness is measured by the 25-week excess mortality rates calculated and published by the Bureau of the Census.[16] These 25-week excess rates indicate the number of people dying from all causes, during the 25 weeks following the initial outbreak of the epidemic in this country in the autumn of 1918, in excess of the number who probably would have died in the same period had no epidemic occurred. The rates for the 34 cities are given in Table I (p. 12) of Influenza Studies I, and hence need not be reprinted here. The values of the other variables used in this paper are given in Table I of Influenza Studies II (p. 9).

III. Demographic and Environmental Correlations.

Just as in the discussion of explosiveness of outbreak, we may consider first the net fifth order correlations of destructiveness with the several demographic and environmental factors for which we have data. The coefficients are exhibited in Table I.

TABLE 1.—*Net correlation of destructiveness (25-week excess mortality) with various demographic and environmental factors.*

Variable correlated with destructiveness (25-week excess mortality).	r subscripts.	Coefficient.
Age distribution of population	24.56789	+0.132±0.111
Sex ratio of population	25.46789	+0.161±0.113
Density of population	26.45789	+0.163±0.113
Latitude of city	27.45689	−0.424±0.095
Longitude of city	28.45679	−0.133±0.111
Rate of growth of population, 1900-1910	29.45678	−0.083±0.115

The results here are clear cut. The only variable having a net coefficient which can be regarded as sensibly different from zero is latitude. There the coefficient is certainly statistically significant. Taken at its face value, and remembering the significance of the negative sign, this means that the farther south the city the greater the rate of mortality in excess of the normal. Or put in another way, the coefficient indicates that the influenza epidemic had a definite, though at best not marked, tendency to be more destructive of life in southern than in northern latitudes. Too much stress must not be laid upon this result, however, because of the peculiar spatial distribution of this group of 34 cities. This point has been fully discussed in the preceding study of this series and need not be

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[16] *Cf.* Public Health Reports, vol. 34, No. 11, p. 505, 1919.

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dwelt on again here. The same reasons exist for suspending judgment as to the full biological significance of the apparently definite correlation between destructiveness of the epidemic and latitude, as were emphasized in the case of the similar correlation between explosiveness of outbreak and latitude.

The remaining coefficients are very definitely so small as to leave no doubt about their meaning. Throughout the long process of making more and more variables constant and getting the successive higher order net coefficients, no one of these demographic characters, except latitude, ever showed a value sensibly differing from zero. This fact is well indicated by the zero order coefficients, which are as follows:

$r_{24} = +0.024 \pm 0.116$ (age).
$r_{25} = -0.029 \pm 0.116$ (sex).
$r_{26} = +0.111 \pm 0.114$ (density).
$r_{27} = -0.325 \pm 0.103$ (latitude).
$r_{28} = +0.0007 \pm 0.116$ (longitude).
$r_{29} = -0.071 \pm 0.115$ (growth).

It can, then, be safely asserted that in the determination of the variation among these 34 large American cities in respect to the excess mortality due to the epidemic, the age and sex distribution of the population, its degree of crowding, its rate of recent growth, or the distance of the city west from the Atlantic seaboard, played no appreciable part whatever. This conclusion is true whether all these factors were allowed to vary together, or whether, as in a laboratory experiment, one to five of them were held constant, while the net influence of one or more varying alone was tested.

Whether or not the same conclusion will hold generally for other cities of a different order of size, or for rural districts, remains to be shown by further work. But as to the facts for the 34 cities listed in Table I of the second of these Studies, there can be no doubt or argument.

IV. Death Rate Correlations.

Turning to the same set of normal death rate variables as were studied in connection with explosiveness we have the results set forth in Table II.

Table II.—*Net correlation of destructiveness (25-week excess mortality) with the normal death rate from certain specified causes.*

Variable correlated with destructiveness (25-week excess mortality). Death rate from—	Subscripts.	Coefficient.
All causes	228. 456789	+0. 405 ± 0. 097
Pulmonary tuberculosis	229. 456789	+0. 279 ± 0. 107
Organic diseases of the heart	230. 456789	−0. 537 ± 0. 082
Nephritis and Bright's disease	231. 456789	−0. 098 ± 0. 115
Typhoid fever	232. 456789	−0. 138 ± 0. 113
Cancer and other malignant tumors	233. 456789	+0. 258 ± 0. 107

From this table we note the following points:

1. Epidemic excess mortality is significantly correlated with the normal death rate from all causes in these cities. This is true in gross ($r_{23f} = +0.435 \pm 0.094$) and also when the environmental and demographic factors listed in Table 1 above are held constant, as in an experiment. In other words, the number of people dying during the epidemic in each of these 34 cities was determined to a significant, though not a high, degree by the usual mortality relations of the community, as indicated by the normal death rate from all causes. Cities which have normally a high death rate had also a relatively high mortality from the influenza epidemic, and *vice versa* those normally enjoying a relatively low mortality rate lost but relatively few persons in the epidemic. It is to be noted, however, that while the net coefficient $r_{23f.456789}$ has a value more than 4 times its probable error and therefore is to be regarded as certainly significant statistically, yet this correlation is lower than the corresponding one for explosiveness of outbreak and death rate from all causes (cf. Table III, Influenza Studies II). We have:

$$\begin{array}{lr} \text{Explosiveness,} \quad r_{13f.456789} = & +0.572 \pm 0.078 \\ \text{Destructiveness,} \quad r_{23f.456789} = & +0.405 \pm 0.097 \\ \hline \text{Difference} & 0.167 \pm 0.124 \end{array}$$

While this difference is not significant in comparison with its probable error, nevertheless there is considerable probability that with a larger sample it would become so. It thus appears that the explosiveness of outbreak of the epidemic mortality was, perhaps, somewhat more influenced by the normal mortality rate of the community than was its total magnitude or destructiveness.

2. The total destructiveness of the epidemic is not significantly correlated with the normal death rate of the community from pulmonary tuberculosis when the important demographic and environmental factors listed in Table 1 are held constant. Here we come upon a distinct break between the two epidemiological characteristics, explosiveness and total destructiveness. The former is, and the latter is not, significantly correlated with the normal tuberculosis death rate. A difference of the same sense is evident in the zero order gross correlations, which are $r_{13a} = +0.578 \pm 0.077$ and $r_{23a} = +0.428 \pm 0.094$. Both of these gross values are more than 3 times the probable error, but owe a considerable portion of their high values, as is demonstrated by the sixth order coefficients, to intercorrelations with other variables.

3. The highest net correlation of destructiveness of the epidemic is with the normal death rate from organic diseases of the heart. When all of the demographic and environmental factors with which

we are dealing are held constant for these cities, we find a correlation of $+0.537 \pm 0.082$, a value nearly 7 times its probable error, between these two variables. The coefficient is higher than that for the normal death rate from all causes with destructiveness. It appears very clearly that of the 12 different factors here studied the normal death rate of the community from organic diseases of the heart had more to do with determining the proportionate part of the population which perished in the epidemic than any other factor. In those cities having normally a high heart-disease rate a relatively large number died in the influenza epidemic, and *vice versa*. The same thing was shown in the preceding study to be true for explosiveness of outbreak of epidemic mortality. The condition of the population in respect to cardiac soundness played a significant rôle in determining the suddenness and frequency with which people died during the autumn and winter of 1918, when the 34 large American cities were struck by the influenza epidemic. It is interesting to note that the net sixth order correlation coefficient is higher in the case of organic diseases of the heart than the gross zero order coefficient, which is $r_{23b} = +0.487 \pm 0.088$. This means that in the gross, or zero order coefficient, the true organic relationship existing between destructiveness of epidemic and normal cardiac death rate is obscured by the fact that there is a high correlation between the latter variable and the age distribution of the population ($r_{3b_1} = +0.609 \pm 0.073$), the meaning of this coefficient being, of course, that the higher the average age of a population, the higher the death rate from organic diseases of the heart, and *vice versa*—a relationship which would be expected *a priori* from what we know about cardiac affections. As soon as we make the cities all constant in respect to age distribution of the population, we get a marked increase in the correlation coefficient between epidemic destructiveness and normal cardiac rate ($r_{23b.1} = +0.596 \pm 0.075$). This is an increase of 0.109 in the coefficient. But, as there is also a sensible negative correlation in this group of 34 cities between normal death rate from organic heart diseases and latitude ($r_{3b.7} = -0.300 \pm 0.105$), the high value of $r_{23b.1} = +0.596 \pm 0.075$ is reduced somewhat when the cities are made constant in respect to both age distribution and latitude, the coefficient being $r_{23b.17} = +0.549 \pm 0.081$. The other demographic and environmental variables have only negligible effect upon the r_{23b} correlation.

4. The net correlation between destructiveness of the epidemic and the normal death rate from diseases of the kidneys is sensibly zero. Here again there is a marked contrast between explosiveness of epidemic mortality and destructiveness (cf. Table III, Influenza Studies II). The gross zero order correlation between destructiveness and normal death rate from kidney diseases ($r_{23c} = +0.282 \pm$

0.106) is less than three times its probable error, as well as the net sixth order coefficient.

5. The destructiveness of the epidemic is not significantly correlated with the normal death rate from either typhoid fever or cancer, either net, when all the demographic and environmental variables are held constant, or in gross ($r_{23d} = +0.014 \pm 0.116$, and $r_{23e} = +0.215 \pm 0.110$). We observe the same contrast in the correlations of these diseases with destructiveness as we did in the previous study, when they were correlated with explosiveness, in comparison with the correlation of normal death rate from organic diseases of the heart with these same epidemiological characteristics.

V. Summary.

In order to meet a justifiable criticism of the earlier work we have determined in this paper the correlations between destructiveness of the influenza epidemic of 1918–19, as measured by the 25-week excess mortality in the 34 cities used in the earlier work, and the series of demographic, environmental, and biological (normal death rate) variables discussed in Studies II. It is found that there is, in the group of large cities, no significant net correlation between destructiveness of the epidemic as above defined, and any demographic or environmental variable, with the exception of latitude. In that case, for reasons fully discussed in Studies II, the biological meaning to be attached to the result is not entirely clear. The highest net correlation between destructiveness and any of the 12 variables discussed was with the normal death rate from organic diseases of the heart. Those cities, within this group of 34, which have normally an unusually high death rate from cardiac disorder, had an unusually high epidemic mortality, and *vice versa*. There was no significant net correlation between destructiveness of the epidemic and the normal death rate from acute nephritis and Bright's disease, nor with that from pulmonary tuberculosis. The correlation results indicate clearly that explosiveness of outbreak of epidemic mortality and total destructiveness of the epidemic are distinct but related epidemological characteristics.

IV. ON THE CORRELATION BETWEEN EXPLOSIVENESS AND TOTAL DESTRUCTIVENESS OF THE EPIDEMIC MORTALITY.[17]

The results of the second and third of these Studies indicate clearly that explosiveness of outbreak of epidemic mortality (as measured by the epidemicity index I_0) and total destructiveness of the epidemic (as measured by the 25-week excess mortality) are distinct epidemiological characters. The question then presents itself as to how closely these two characters are correlated. To the answer of this question the present paper is addressed.

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[17] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University. No. 23.

For the 34 large American cities furnishing the material set forth in Table I of the second of these Studies,[13] we have data on the following variables:

Subscript No.	Variable.
1.	Explosiveness of outbreak of epidemic mortality, I_6.
2.	Destructiveness (25-week excess mortality).
3a.	Normal death rate from pulmonary tuberculosis.
3b.	Normal death rate from organic heart diseases.
3c.	Normal death rate from acute nephritis and Bright's disease.
3d.	Normal death rate from typhoid fever.
3e.	Normal death rate from cancer and other malignant tumors.
3f.	Normal death rate from all causes.
4.	Age distribution of population.
5.	Sex ratio of population.
6.	Density of population.
7.	Latitude.
8.	Longitude.
9.	Rate of growth of population, 1900-1910.

The gross or zero order correlation between explosiveness of epidemic mortality (I_6) and total destructiveness (25-week excess mortality) is

$$r_{12} = +0.709 \pm 0.058.$$

This obviously represents a relatively high, but by no means perfect, correlation. In general, it means that cities having an incidence of epidemic mortality more sudden and explosive in its outbreak than the average were highly likely to have a total mortality from the epidemic above the average, and *vice versa*.

It is essential, however, just as in earlier parts of these Studies, to find the *net* value of this correlation when the various environmental, demographic, and biological variables listed at the beginning of this paper are held constant as in an experiment. The initial step in such a process is to calculate the first-order coefficients, where successively, one at a time, each variable in the series is held constant while the correlation between variables 1 and 2, in which we are interested, is determined.[19] In Table 1 are given these first-order correlation coefficients between the variables 1 and 2 (explosiveness and destructiveness).

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[13] See p. 9.

[19] For a general discussion of the method of multiple correlation in statistical work of this sort the reader is referred to these Studies, II, pp. 3-5.

TABLE 1.—*First-order correlations between explosiveness (I_6) and destructiveness (25-week excess mortality) of the 1918 influenza epidemic in 34 American cities.*

r subscripts.	Coefficient.
12.3a	+0.626±0.070
12.3b	+0.592±0.075
12.3c	+0.679±0.062
12.3d	+0.750±0.051
12.3e	+0.694±0.060
12.3f	+0.626±0.070
12.4	+0.718±0.056
12.5	+0.736±0.053
12.6	+0.707±0.058
12.7	+0.687±0.061
12.8	+0.729±0.054
12.9	+0.723±0.055

It is evident from this table that making any single one of the 12 variables constant has little effect upon the correlation between explosiveness and destructiveness of the epidemic mortality. The coefficients are all relatively high.

Let us examine the effect of making *all* the demographic and environmental factors (subscripts 4 to 9, inclusive) constant at the same time. The coefficient is

$$r_{12.456789} = +0.706 \pm 0.058.$$

This is almost absolutely identical with the zero order gross coefficient given above. This result means that the factors age and sex distribution, density and rate of recent growth of the population, latitude and longitude of the city (with all implied climatic differences), have no influence in determining the correlation between explosiveness and total excess mortality rate.

Taking in the biological (normal death rate) variables we have the following seventh-order coefficients:

$$r_{12.3a456789} = +0.675 \pm 0.063$$
$$r_{12.3b456789} = +0.579 \pm 0.077$$
$$r_{12.3c456789} = +0.711 \pm 0.057$$
$$r_{12.3d456789} = +0.749 \pm 0.051$$
$$r_{12.3e456789} = +0.700 \pm 0.059$$
$$r_{12.3f456789} = +0.632 \pm 0.069$$

It is obvious that these normal death rates influence very little, either one way or the other, the correlation between explosiveness and destructiveness of the epidemic outbreak.

One point of considerable interest which attaches to the relatively high correlation between these two variables is that one of the variables is measurable in time long before the value of the other can be possibly determined. The value of the explosiveness index I_6 can usually be determined in from 2 to 4 weeks after the beginning of the epidemic, while the total excess mortality can only be measured when the epidemic is all over. With a correlation coefficient of the magnitude of r_{12} above, one can, by means of the regression equation, make a very fair prediction of the total excess mortality rate from a knowledge of the explosiveness of outbreak of the mortality measured by L_1. Actually, as the different net correlations show, we shall get practically as good a result by using the zero order coefficient and a simple equation of the form $y = a + bx$ as by employing a many-con-

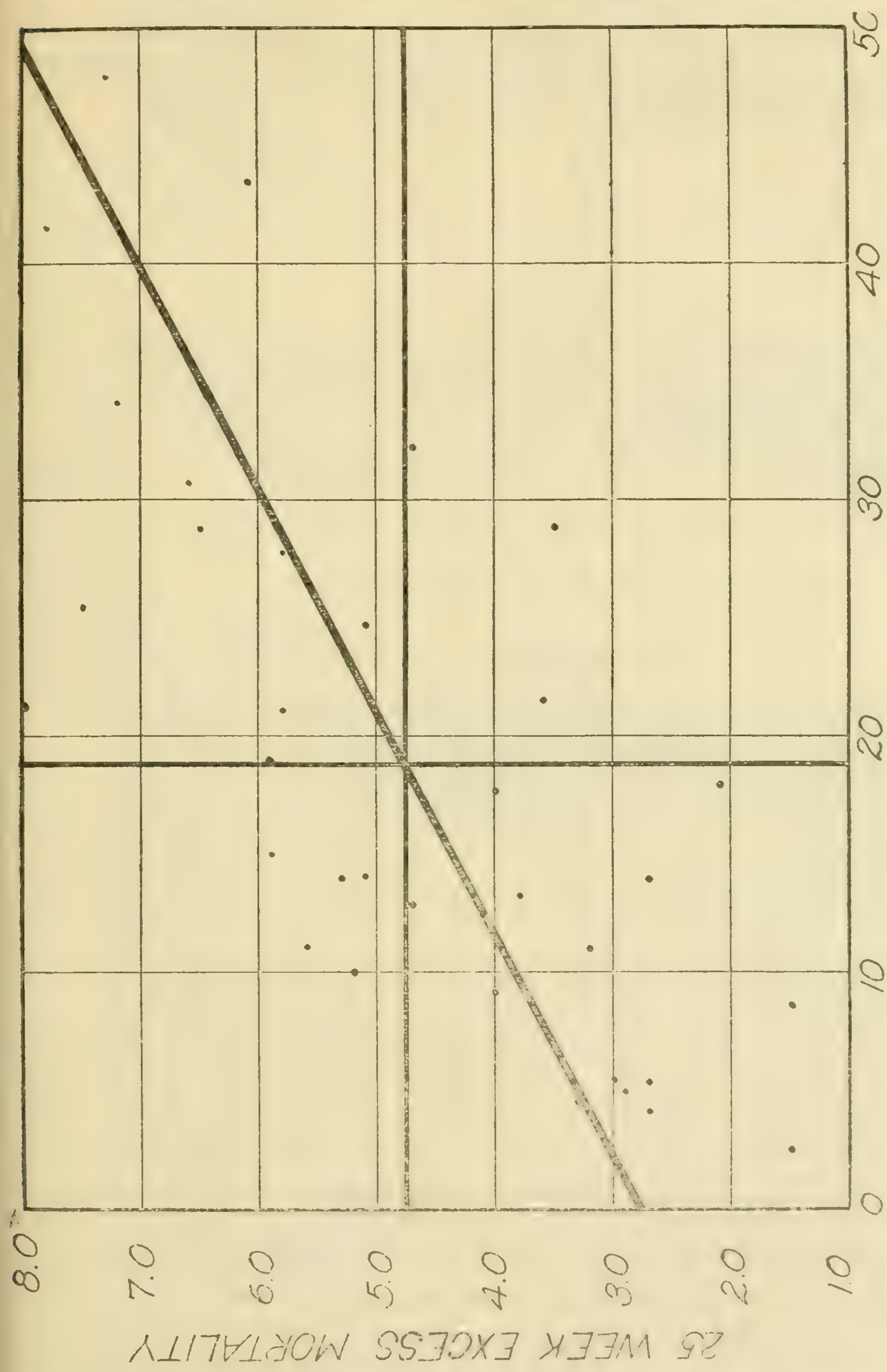

FIG. 2. Regression line of 25-week excess mortality rate of influenza epidemic on explosiveness of outbreak of epidemic mortality.

stant partial regression equation. Actually the regression equation from the zero order r_{12} is

$$D = 2.7412 + 0.1065\ I_6$$

where D denotes 25-week excess mortality rate and I_6 is the epidemicity or explosiveness index. This regression line is shown graphically in Figure 2.

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[Reprinted from THE AMERICAN JOURNAL OF HYGIENE, Vol. I., No. 3, May, 1921.]

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE DOMESTIC FOWL. XIX. ON THE INFLUENCE OF FREE CHOICE OF FOOD MATERIALS ON WINTER EGG PRODUCTION AND BODY WEIGHT.*

BY RAYMOND PEARL AND THOMAS EVERETT FAIRCHILD.

(Received for publication, January 20th, 1921.)

INTRODUCTION.

From extensive and careful observation of the behavior of the domestic fowl the senior author has long held the opinion that current practice in the feeding of poultry is, from a theoretical point of view at least, biologically unsound. The methods of feeding poultry advocated by the recognized authorities in that branch of husbandry are essentially mass or average methods, while appetite and vital performance of whatever sort are biologically highly individualistic matters. Some years ago the following statement was published regarding the matter (1): "The successful feeding of poultry depends upon experience and acquaintance with fowls. The basic biological factor is, once more, individuality. Each individual hen is an independent living thing, possessing well marked likes and dislikes of her own with respect to food. There can be no question that the best results in the way of egg production and meat production would be obtained if a skillful feeder could feed each individual fowl by and for itself. Evidence that this is the case is found in the fact, which is universal wherever poultry is kept, that on the average fowls kept in small flocks, of say under 25 birds each, do relatively much better than larger flocks. The production and money returns per bird are greater. The fundamental reason for this is that the birds in small flocks get better care as individuals. When a man has only such a small number to take care of he can recognize their individual peculiarities more easily. Furthermore an individual bird stands a better chance of having its peculiar taste gratified in a small than in a large flock."

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* Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 25.

The experiments here recorded were carried out at the Maine Agricultural Experiment Station during the autumn and winter of 1916–17. The entry of the United States into the war broke off the work. It has been completed and prepared for publication by the senior author.

It may be that mass or average methods of feeding poultry are economically preferable to individualistic methods, but there is no real evidence that such is the fact, for the simple reason that no adequate data on the point have ever been collected. Such experiments as have hitherto been made in feeding poultry in ways which would permit of free individual choice of food materials on the part of the birds have dealt with small numbers.

The purpose of the present investigation was to determine, with a sufficiently large number of birds, to give a statistically significant result, and selected with extreme care to secure genetic uniformity between controls and experimental birds, what effect on fecundity would be produced by permitting birds free choice as to kind and amount of food eaten, in comparison with others fed by the usual mass methods. Incidentally it was possible to make observations on a number of other interesting points.

Pioneer work in the field of animal husbandry on the subject of free choice feeding was done by Evvard (2) with hogs. His first experiment was to give the pigs an opportunity to choose the amount of salt to be used in their diet. A basal ration was fed to five lots of pigs and after a check period of ninety days, one lot was allowed no salt; the second lot received 18 grams of salt daily; the third lot received 36 grams of salt daily; and the fourth lot received 72 grams of salt daily. In each of the above instances where salt was fed it was mixed with the basal ration "of 6.1 parts corn to 1 part linseed oilmeal." The fifth pen received the basal ration, but the pigs were allowed free access to the salt. The results of this experiment in Evvard's own words were:

"The requirement for a hundred pounds of grain was comparatively less in the 'salt ad libitum' group (Lot V) than where the salt was omitted (Lot I), or mixed with the feeds (Lots II, III, and IV). It is quite evident that the addition of salt in varying results, in every instance, is a cheapening of the gain; the feed required for a hundred pounds was lessened. It is quite evident that the pigs which received salt according to their appetite made relatively the most economical gains of any group. The free choice group compared to the 'no salt group' in the 71-day salt feeding period required only 89.36 in contrast with 101.88 per cent. as much feed as in the preliminary."

In the second experiment " three groups of five pigs each were fed in dry lot practically from weaning time until they were more than eight months of age." In each pen the pigs were allowed to select their diet and to eat as much as they wanted, and to eat at any time they wished, for food was always before them. A careful record was kept of the food consumed, and the ration eaten compared to rations compounded according to the " Illinois or Dietrich Standard " and the " Wolff-Lehmann standard."

Among the very interesting points brought out by the above experiment are:

1. That a " very marked changeableness of the pigs' appetites is manifested by what they eat throughout the period of growth and fattening." During the growing period the young pig consumes a larger amount of " high protein feeds," but when growth slackens the desire changes and the pig consumes a greater amount of the " carbohydrate-fat " feeds; or to put it in another way " as the pigs unfold in their development they vary their intake of food nutrients."

2. In the second place, greater development was brought about by the " free-choice " method of feeding than has been secured by any other method of feeding.

3. It would seem that the palatability of the foods varied with the degree of development in the animal.

Jackson (3) performed an experiment of this sort with poultry. The hens were kept in separate pens, one individual by itself in each pen, and fed foods placed in separate holders. The results of the three experiments reported show a marked individual preference of food on the part of the birds used. In the first experiment the foodstuffs consumed in the largest amount were as follows, named in the order of decreasing amounts: Wheat, corn, oats, mash; the second experiment showed a different order—namely: Wheat, corn, mash, oats; and the third experiment showed still a third order: Corn, wheat, oats, mash. On examination of the figures given it is seen that in the third experiment the wheat was only slightly less in amount than the corn consumed. The other two experiments conformed closely to the results here reported.

A similar experiment to that of Jackson was carried on by Kempster (4). He used only sixteen hens, each one having a separate pen, and with the food placed before them in cans. The purpose of the experiment was, among other things, to " study individual preference for certain foods " and " to observe changes in their food selection while laying and when not."

It was found that wheat was preferred above all the other food presented, the birds eating more than two and a half times as much wheat as any other one grain. There was noticed, however, a variation in the tastes of the individual birds, for " five consumed more kafir " than wheat; " one consumed more middlings;" while another consumed " more corn meal," which together with Evvard's experiment indicates that the system of feeding a ration made up of only one or two kinds of food to a group of animals or a flock of hens is utterly insufficient to meet the needs of all of the individuals which go to make up the group or flock. It was also found that the birds when laying consumed a ration having a narrower nutritive ratio than when they were not producing eggs.

MATERIAL AND METHODS.

The experiment began September 21, 1916, when the pullets were taken from the range to winter quarters in the long laying house. In order that all conditions, save the food, should be the same one of the large compartments of the laying house was divided by a wire partition. The litter on the floor throughout the entire experiment was made up of soft wood shavings.

The two pens were designated as A and B. Pen A was known as the experimental pen and Pen B as the check pen, and the birds in the latter were given the regular Maine Station ration. Great care was taken, in selecting the pullets, to have those in Pen B as nearly identical to those in Pen A as was possible. In making the selection an equal number of birds accustomed to a given chick ration were placed in each pen, and when a new breed or cross-bred combination was introduced an equal number of individual representatives of the breed was placed in each pen.

On September 21, 1916, 25 pure bred Barred Plymouth Rock pullets hatched April 2, 1916, were put in Pen A, and a like number in Pen B. Four pullets* were added to each pen on October 10. The next

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* For exact information as to breeds see Table I.

TABLE I.

Showing date, number and breed of birds put in Pens A and B.

Pen A.		Date.	Pen B.	
No. of birds.	Breed.		No. of birds.	Breed.
25	B. P. R.	Sept. 21	25	B. P. R.
2	B. H. × Wh. Leg.	Oct. 10	1	B. H. × Wh. Leg.
2	B. P. R. × B. H.	Oct. 10	3	B. P. R. × B. H.
9	B. P. R. × Hdn.	Oct. 11	9	B. P. R. × Hdn.
1	B. P. R. × Hdn. × B. H.	Oct. 11	1	B. P. R. × Hdn. × W.
6	B. P. R. × B. H.	Oct. 11	5	B. P. R. × B. H.
2	Golden Barred	Oct. 11	2	Golden Barred
1	Extracted White	Oct. 11	1	Extracted White
1	W. W. × E. W.	Oct. 11	1	B. P. R.
1	B. P. R. × Hdn.	Oct. 12	1	B. P. R. × Hdn.
1	W. P. R.	Oct. 12	1	W. P. R.
1	W. W.	Oct. 12	1	W. W.
1	B. P. R.	Oct. 12		
1	Bh. × Wh. Leg.	Oct. 20	1	B. B. × Wh. Leg.
1	B. P. R. × B. H.	Oct. 20	2	B. P. R. × B. H.
2	B. H. × Wh. Leg.	Nov. 7	3	B. H. × Wh. Leg.
2	B. P. R. × B. H.	Nov. 7	2	B. P. R. × B. H.
2	B. P. R.	Nov. 11	2	B. P. R.
61			61	

Key to above table:
B. P. R. = Barred Plymouth Rock.
B. H. = Black Hamburg
Wh. Leg. = White Leghorn
Hdn. = Houdan
W. W. = White Wyandotte
E. W. = Extracted White
Hdn. W. = White Houdan

day, October 11, twenty pullets were put in Pen A and nineteen in Pen B, and the following day, October 12, four were added to Pen A and three to Pen B. On October 20 two pullets were added to Pen A and three to Pen B. The only loss from death occurred on October 28 when No. 296 from Pen A died, death being caused by a pathological condition of the ovary. In November 7 three pullets were added to Pen A and five to Pen B, and on November 11 two birds were added to each pen, making a total of 60 birds in Pen A and 61 in Pen B or 121 birds used throughout the experiment.

The following materials were given as food:

Pen A.

Wheat,
Cracked corn,
Oats,
Bran,
Red-dog flour,
Linseed meal,
Bone meal,
Meat scrap,
Shell,
Grit,
Charcoal,
Green food.*

Pen B.

Cracked corn,
Wheat,
Oats,
Dry mash,†
Shell,
Grit,
Green food.*

Bran	300 lbs.	Middlings	100 lbs.
Cornmeal	100 lbs.	Meat scrap	100 lbs.

The composition of the mash varied each month as follows:

From September 1 to October 1:

Bran	300 lbs.	Middlings	100 lbs.
Cornmeal	100 lbs.	Meat scrap	100 lbs.

From October 1 to November 1:

Bran	200 lbs.	Middlings	100 lbs.
Cornmeal	100 lbs.	Meat scrap	100 lbs.

From November 1 to December 1:

Bran	200 lbs.	Gluten meal	100 lbs.
Cornmeal	100 lbs.	Meat scrap	100 lbs.
Middlings	100 lbs.	Linseed meal	50 lbs.

From December 1 to January 1:

Bran	200 lbs.	Gluten meal	100 lbs.
Cornmeal	100 lbs.	Meat scrap	100 lbs.
Middlings	100 lbs.		

From January 1 to March 1:

Bran	200 lbs.	Gluten meal	100 lbs.
Cornmeal	100 lbs.	Meat scrap	100 lbs.
Middlings	100 lbs.	Linseed meal	50 lbs.

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* The green food varied from time to time as various materials were available. From September 21 to October 9 the green food was in the form of cut corn; from October 9 to December 10 cabbage was given; from December 10 to December 13 mangel beets were used; and from December 13 to the close of the experiment sprouted oats were given.

† The dry mash given was the regular Maine Experiment Station dry mash consisting of:

In Pen A the foods except the green food were placed in separate compartments of specially built hoppers, which were kept well filled so that the hens had access to food at all times of the day. The green food when in the form of cut corn and sprouted oats was thrown on the floor, and when in the form of cabbage and mangels was hung on a nail within easy reach of the pullets. After the hoppers had once been put in place they were never changed and throughout the experiment they contained the same kind of food. All of the hoppers used were of the same size, and with one exception, were divided into two equal compartments each holding a different kind of food. One hopper was divided into three compartments and held the grit, oyster shell and charcoal in the respective parts.

In Pen B the green food was fed in the same manner as in Pen A. The wheat, oats and cracked corn, mixed together, were given as a scratch food and thrown in the litter twice a day, morning and at noon. The dry mash was fed in a long hopper which was filled every morning. The grit and oyster shells were mixed and offered in one hopper during the first part of the experiment, but after November 10 they were fed in separate hoppers. The charcoal was mixed with the dry mash.

An accurate record was kept of all the food that was put into the hoppers, and after the first period, which was from September 21 to October 21 inclusive, the hoppers were cleaned every Friday morning. All of the material found in the hoppers at that time was carefully weighed, and the amount subtracted from the total amount put in during the week gave the amount consumed by the hens during that period. After the hoppers had been cleaned fresh food was placed in them so that after the first period the birds received fresh food every Friday morning. This procedure reduced the chance of the food becoming mouldy or stale.

During the first period birds were being added from time to time, and consequently they were in a more or less disturbed state, and time was allowed for them to become accustomed to the new environment.

Every twenty-eight days the birds were weighed individually and the measurement of the width of the pubic bones taken. In this way we were able to acquaint ourselves with the monthly gain or loss of each bird.

At the end of each feeding period the data collected were arranged in suitable tables and the net amount of food consumed, as well as the number of grams of food consumed per bird per day; the number of grams of food used in the production of one egg, and the percentage of each food eaten to the total amount consumed were determined.

In determining the nutritive ratio, the ration made up of the food eaten during each period was used as a basis, and not the amounts eaten each day. In determining the nutritive ratio of the ration eaten by Pen B in which the dry mash composed the larger part it was first necessary to find the sum of the respective digestible nutrients of the several constituents of the mash. As we were unable to find any publications giving the digestion coefficient for sprouted oats it was necessary to use, in determining the nutritive ratio of the ration, the digestible nutrients of whole oats. The percentages of available nutrients used for sprouted oats were determined by Mr. James M. Bartlett, Chemist of the Maine Experiment Station, especially for this work, using oats having sprouts about four inches long. In making the determination the entire plant, both stem and roots, was used. We are greatly indebted to Mr. Bartlett for this aid.

In figuring the nutritive ratio of the various rations the following formula was used:

$$\frac{\text{Carbohydrates} + \text{Fat} \times (2.25)}{\text{Protein}}.$$

It will be seen that the fiber was not taken into consideration.

In the case of food materials fed from hoppers it is possible to determine accurately the amount actually consumed by the birds, because one can weigh in an amount at the beginning of a period, weigh out what is left at the end, and the difference is the amount consumed. The hoppers were so constructed as to prevent the birds scattering and wasting the food in the process of feeding from them. In the case of free-choice Pen A the consumption of all materials fed except green food could be accurately determined in this way. In the case of Pen B the only materials for which accurate consumption figures could be got in this way were dry mash, shell and grit. The whole grains, wheat, oats, and cracked corn, were in Pen B, as already explained, scattered on the litter and the birds scratched for them. Consequently the figures in that case, just as in all statistical estimates of human food consumption (5) represent consumption plus undeterminable waste. Careful as the feeder may be there will always be a certain amount of grain left in the litter when fed in this way.

In the case of green food the same condition obtained in both pens. When the green food is in the form of cabbage or mangel-wurzel the consumption can be accurately determined, because the uneaten residue can be weighed. When sprouted oats are used, however, a certain undeterminable amount gets trampled in the litter and lost. So that here again figures given represent consumption plus wastage. In respect of this food, however, both pens were on precisely the same footing.

FOOD CONSUMED.

In Tables II and III are set forth the amounts of each kind of food consumed in each period by each pen of birds.

TABLE II.

Net kilograms of food consumed in each period. Pen A.

Period.	Wheat.	Cracked corn.	Oats.	Bran.	Flour.	Corn-meal.	Lin-seed meal.	Bone-meal.	Meat Scrap.	Shell.	Grit.	Char-coal.	Green food.
1....	95.91	29.55	20.80	6.06	.61	13.59	.00	1.00	11.54	4.45	1.84	1.63	134.49
2....	22.00	12.38	4.31	.11	.48	2.45	.05	.59	1.22	.88	.50	.05	30.05
3....	21.64	9.32	4.76	2.86	.39	3.72	.00	.50	1.86	.86	.84	.09	29.48
4....	21.82	8.98	4.58	.64	.39	5.17	.00	.32	1.81	.95	.61	.00	24.95
5....	19.69	7.08	3.63	.64	.50	5.22	.00	.07	1.72	.86	.91	.05	25.51
6....	21.95	6.42	3.08	.91	.64	4.97	.00	.27	1.63	1.04	.91	.02	17.46
7....	21.41	7.80	5.83	1.18	.54	4.60	.00	.14	1.86	1.02	.73	.05	17.69
8....	26.97	8.23	8.12	.57	.66	5.35	.00	.18	1.22	1.13	.88	.02	16.06
9....	19.96	8.89	7.89	.64	.41	3.63	.00	.25	1.02	.95	1.09	.05	14.52
10....	17.37	12.93	8.98	1.09	1.13	2.27	.00	.27	2.09	1.22	1.00	.05	9.19
11....	19.82	11.07	10.05	.64	.91	5.33	.00	.09	1.66	1.32	.82	.09	11.34
12....	19.16	10.36	8.03	.54	.43	6.26	.00	.05	1.77	1.27	1.52	.09	12.81
13....	23.07	8.75	5.53	.54	.61	4.63	.00	.00	1.27	1.66	.86	.07	12.47
Totals	350.77	141.76	95.59	16.42	7.70	67.19	.05	3.73	30.67	17.61	12.51	2.26	356.02

TABLE III.

Net kilograms of food consumed in each period. Pen B.

Period.	Cracked corn.	Wheat.	Oats.	Dry mash.	Shell.	Grit.	Green food.
1..........	50.35	15.24	18.69	100.15		5.08	122.79
2..........	12.70	7.62	5.08	27.67		.59	25.51
3..........	12.70	7.62	5.08	27.01		.95	21.55
4..........	12.70	7.62	5.08	27.13	1.45	1.95	16.67
5..........	12.70	7.62	5.08	27.49	1.04	1.41	18.48
6..........	12.70	7.62	5.08	29.39	1.18	3.18	13.83
7..........	12.70	7.62	5.08	29.39	1.18	1.18	13.38
8..........	12.70	7.62	5.08	32.39	.59	2.04	13.38
9..........	12.70	7.62	5.08	30.21	1.09	1.07	14.06
10..........	12.70	7.62	5.08	27.13	1.72	1.13	10.09
11..........	12.70	7.62	5.08	29.21	1.72	1.63	11.34
12..........	12.70	7.62	5.08	28.21	1.81	.54	12.81
13..........	12.70	7.62	5.08	26.76	1.68	1.32	12.93
Totals.....	202.75	106.68	79.65	442.14	13.46	22.07	306.82

For these tables the following points are to be noted:

1. Considering first the whole grains, the " free-choice " birds ate more than three times as much wheat as the mass-fed birds ate and wasted; the former also ate somewhat more oats than the total consumption and wastage of the latter. Of cracked corn the consumption and wastage of the mass-fed birds was greater than the consumption of the "free-choice" birds. Taking totals of all "scratch-feeds" (whole grains) the Pen A (free-choice) birds ate during the whole experiment 588.12 kgs. against 389.08 kgs. of the same feeds consumed and wasted by the Pen B (mass-fed) birds.

2. The reverse relation obtained in respect of ground materials (mash). In total Pen A (free-choice) birds consumed during the whole experiment 128.02 kgs. (including bran, red-dog flour, cornmeal, linseed meal, bone meal, meat scrap, and charcoal) against 442.14 kgs. of the same materials in the form of dry mash consumed by the Pen B birds. In other words, when the birds were given free choice of their food about 12 per cent. of the ration was made up of milled products (including meat and charcoal) while the mass-fed birds took about 38 per cent. of their total ration in this form.

3. A noteworthy point is the small amount of beef scrap eaten by the " free-choice " birds. It has often been observed that birds eating a dry mash in which beef scrap was a constituent part, would pick out the bits of meat, and it was expected that birds having free access to beef scrap would avail themselves of the opportunity and eat a large amount. This, however, was not the case, for the total amount of beef scrap eaten amounted to 30.67 kilograms which is 2.78 per cent. of the ration. This observation is similar to that noted by Jackson (*loc. cit.*) who states: "The low consumption of meat scrap was something of a surprise. None of the hens in any of the tests . . . displayed any particular liking for this foodstuff, though the brand used was considered the most palatable that could be obtained." Kempster (*loc. cit.*), likewise found that the birds when allowed to choose their own ration ate only a small amount of beef scrap, and concluded that " the comparatively small consumption of beef scrap indicates that hens eat only enough to satisfy their demands."

4. The birds in Pen B consumed more grit and oyster shell than the "free-choice" birds, even though they were fed, in this respect, in nearly the same way. Except for the first three periods, grit and oyster shell were fed in separate hoppers, as in Pen A. During the

first three periods the grit and shell were mixed and fed in the same hopper. It will be noticed that the total amount of grit and shell consumed in Pen B is 35.53 kgs. while the "free-choice" birds ate only 30.12 kgs. The difference in consumption of these ingredients is probably correlated with the differences already noted in the composition of the grain portion of the rations consumed.

5. The amount of green food eaten shows that the "free-choice" birds consumed somewhat more than the birds in Pen B. The green food was measured out to both pens in nearly the same amounts at first, but it was noticed that the "free-choice" birds ate the green food much more eagerly and apparently with greater relish, so that the amount was slightly increased. They consumed in total 256.02 kgs., which is 32.30 per cent. of all they ate, while the birds in Pen B consumed 306.82 kgs., which is 26.14 per cent. of the total consumption of that pen.

6. Although bran is considered as a very necessary part of all rations fed to poultry, the "free-choice" birds ate very little—only 16.42 kgs., which is less than one per cent. of the total amount. Only a very small amount of red-dog flour was eaten, due probably to the fact that as soon as it is taken into the mouth it forms a dough which is swallowed only with difficulty. Cornmeal made up 6.10 per cent. of the ration, but bone meal was less than one per cent.

TABLE IV.

Per cent. of total amount of food consumed. Pen A.

Period.	Wheat.	Cracked corn.	Oats.	Bran.	Flour.	Cornmeal.	Linseed meal.	Bone meal.	Meat scrap.	Shell.	Grit.	Charcoal.	Green food.
1..	29.83	9.19	8.47	1.88	.19	4.23	0	.27	3.59	1.38	.57	.51	41.83
2..	29.31	16.49	5.74	.15	.63	3.26	.06	.79	1.63	1.18	.66	.06	40.03
3..	29.19	12.07	6.17	3.70	.50	4.82	0	.65	2.41	1.12	1.09	.012	38.18
4..	31.07	12.79	6.52	.90	.05	.74	0	.05	2.58	1.36	.87	.0	35.53
5..	29.88	10.74	5.51	.96	.76	7.92	0	.10	2.62	1.31	1.38	.07	38.74
6..	37.02	10.82	5.20	1.53	1.07	8.37	0	.46	2.75	1.76	1.53	.04	24.44
7..	34.06	12.41	9.27	1.87	.87	7.33	0	.22	2.96	1.62	.12	.07	28.14
8..	38.85	11.86	11.69	.82	.96	7.71	0	.26	1.76	1.63	1.27	.03	23.13
9..	33.66	14.99	13.31	1.07	.68	6.12	0	.43	1.72	1.60	1.83	.07	24.48
10..	30.16	22.46	15.59	1.89	1.97	3.94	0	.47	3.62	2.13	1.73	.08	15.95
11..	31.40	17.53	15.92	1.01	1.44	8.44	0	.14	2.62	2.08	1.29	.14	17.96
12..	30.76	16.63	12.88	.87	.69	10.04	0	.07	2.84	2.03	2.43	.14	20.56
13..	38.78	14.72	9.30	.92	1.82	7.78	0	0	2.13	2.78	1.45	.10	20.98

The percentages of total consumption for the two groups are shown in Tables IV and V.

TABLE V.

Per cent. of total amount of food consumed. Pen B.

Period.	Cracked corn.	Wheat.	Oats.	Dry mash.	Shell.	Grit.	Green food.
1	16.12	4.88	5.98	32.07		1.63	39.32
2	16.04	9.63	6.42	34.95		.74	32.23
3	16.95	10.17	6.78	36.06		1.27	28.76
4	17.47	10.49	7.00	37.36	2.00	2.68	22.96
5	17.20	10.32	6.88	37.23	1.41	1.90	25.03
6	17.40	10.44	6.96	40.27	1.62	4.35	18.95
7	18.00	10.80	7.20	41.67	1.67	1.67	18.97
8	17.20	10.32	6.88	43.88	.80	2.76	18.13
9	17.68	10.61	7.07	42.05	1.54	1.48	19.58
10	19.39	11.63	7.76	41.42	2.63	1.72	15.41
11	18.32	10.99	7.33	42.14	2.48	2.36	16.36
12	18.46	11.07	7.38	41.01	2.64	.79	18.63
13	18.65	11.19	7.46	39.31	2.46	1.93	18.98

TABLE VI.

Net kilograms of digestible nutrients consumed in each period. Pen A.

Period.	Protein.	Carbohydrate.	Fat.	Calories.
1	21.013	110.022	6.867	601107
2	4.685	25.097	1.299	134187
3	5.229	26.597	1.246	142074
4	4.868	25.992	1.349	139072
5	4.387	23.049	1.193	123583
6	4.345	23.298	1.267	125119
7	4.673	24.922	1.320	133615
8	5.166	28.166	1.461	150249
9	5.803	28.983	2.019	161399
10	5.427	27.315	1.997	152814
11	6.025	33.822	2.108	182977
12	5.691	33.224	2.111	179184
13	5.336	32.128	1.831	170631
Totals	82.648	442.615	26.068	2396011

TABLE VII.

Net kilograms of digestible nutrients consumed in each period. Pen B.

Period.	Protein.	Carbohydrate.	Fat.	Calories.
1	26.358	91.679	8.452	562555
2	7.737	25.656	2.297	158273
3	7.980	25.163	2.278	157072
4	7.673	24.976	2.278	155046
5	7.782	23.299	2.298	148803
6	8.057	25.593	2.390	160192
7	7.784	25.703	2.353	159180
8	8.358	27.670	2.573	171644
9	8.879	32.693	3.044	198754
10	7.972	29.821	2.697	180033
11	8.612	30.790	2.884	188369
12	8.555	31.023	2.903	189268
13	8.388	30.826	2.881	187571
Totals	124.135	424.892	39.328	2616760

We may next consider the nutrient composition of the rations consumed. The data are given in Tables VI, VII, VIII, IX, and X.

TABLE VIII.

Grams of digestible nutrients per bird per day. Pen A.

Period.	Protein.	Carbohydrate.	Fat.	Calories.
1	15.43	80.78	5.04	441.33
2	12.36	66.22	3.42	353.98
3	13.41	68.20	3.19	364.27
4	11.64	62.18	3.23	332.70
5	10.45	54.88	2.84	294.27
6	10.34	55.47	3.02	297.91
7	11.13	59.34	3.14	318.13
8	12.30	67.06	3.48	357.74
9	13.82	69.01	4.81	384.34
10	12.92	65.03	4.75	363.77
11	14.35	80.53	5.02	435.69
12	13.55	79.10	5.03	426.64
13	12.83	77.23	4.40	410.17
Totals	164.53	885.03	51.37	4780.94

TABLE IX.

Grams of digestible nutrients per bird per day. Pen B.

Period.	Protein.	Carbohydrate.	Fat.	Calories.
1	19.70	68.52	6.32	420.48
2	20.47	67.87	6.07	418.65
3	20.30	64.03	5.80	399.69
4	18.05	58.76	5.36	364.77
5	18.66	54.56	5.38	350.24
6	18.87	59.94	5.59	375.11
7	18.23	60.19	5.51	372.77
8	19.57	64.80	6.02	401.90
9	20.79	76.56	7.13	465.44
10	18.67	69.84	6.31	421.57
11	20.17	72.11	6.75	441.12
12	20.04	72.65	6.79	443.18
13	19.65	72.19	6.74	439.23
Totals	253.17	862.02	79.77	5314.15

From these tables it is evident that:

1. Not only did the "free-choice" birds consume wholly different proportions of the several sorts of foodstuffs, but the net nutrient composition of their ration was widely different from that of the ration consumed by the mass-fed controls.

2. The general nature of the difference in rations may best be indicated by taking the results of the experiment as a whole. The Pen A birds consumed in the whole period 33.4 per cent. *less* protein and 33.7 per cent. *less* fat than did the Pen B birds. The carbohydrate consumption was 4.2 per cent. *more* in Pen A than in Pen B. Finally,

TABLE X.

Nutritive ratios of rations consumed.

Period.	Nutritive ratios. Pen *A*.	Pen *B*.
1	1:6.0	1:4.2
2	1:6.0	1:4.0
3	1:5.6	1:3.8
4	1:6.0	1:3.9
5	1:6.0	1:3.7
6	1:6.0	1:3.8
7	1:6.0	1:4.0
8	1:6.1	1:4.0
9	1:5.8	1:3.9
10	1:5.9	1:4.5
11	1:6.4	1:4.3
12	1:6.7	1:4.4
13	1:6.8	1:4.4

the total energy intake in calories of the Pen A birds was 8.4 per cent. lower than that of controls in Pen B. It need not be emphasized that these differences in the two rations in respect to protein, fat, and calories, are relatively large.

3. In terms of nutritive ratio the birds of Pen A operated on approximately a 1:6 ratio against a ratio of 1:4 in Pen B. Put in another way, in the case of Pen A birds about 15 per cent. of the ration was protein, about 80 per cent. carbohydrate, and about 4.5 per cent. fat, against about 21 per cent. protein, 72 per cent. carbohydrates, and 6.5 per cent. fat in the case of Pen B birds.

4. The above results are clearly not of an accidental average character but the differences in ration between the two sets, which have been pointed out, were maintained with marked consistency and regularity in each of the 13 periods. Indeed, the general smoothness and uniformity of the results is noteworthy and bears evidence to the critical care with which the experimental work was done.

5. In general, the nutritive intake tended to increase during the last half dozen periods. This is in accordance with expectation in view of the cold weather and increasing egg production.

With such extreme differences in nutrient intake one would naturally expect comparable differences in energy and material output in the form of eggs, and in body weight changes. We may next examine the facts in regard to these matters.

EGG PRODUCTION.

The basic data regarding the egg production of these birds are given in Table XI, and are summarized in Table XII.

TABLE XI.

Showing daily egg production and the number of eggs per 100 birds per day.

Date.	Pen A.		Pen B.	
	Daily egg production.	No. of eggs per 100 birds per day.	Daily egg production.	No. of eggs per 100 birds per day.
September 20...	0	0	0	0
21...	4	16	8	32
22...	4	16	9	36
23...	6	24	6	24
24...	10	40	7	28
25...	7	28	5	20
26...	12	48	6	24
27...	6	24	7	28
28...	5	20	6	24
29...	8	32	5	20
30...	10	40	9	36
October 1...	8	32	8	32
2...	7	28	4	24
3...	8	32	8	32
4...	7	28	8	32
5...	8	32	10	40
6...	11	44	8	32
7...	9	36	4	16
8...	10	40	9	36
9...	14	56	12	48
10...	10	34.5	5	17.2
11...	10	20.4	10	20.8
12...	14	26.4	10	19.6
13...	11	20.7	11	21.6
14...	14	26.4	13	25.5
15...	12	22.5	13	25.5
16...	11	20.7	12	23.5
17...	15	28.3	15	29.4
18...	13	24.5	13	25.5
19...	11	20.7	8	15.7
20...	15	27.3	14	25.9
21...	14	25.5	13	24.1
22...	10	18.2	15	27.8
23...	7	12.7	10	18.5
24...	16	29.1	19	35.2
25...	15	27.3	13	24.1
26...	14	25.5	11	20.4
27...	16	29.1	15	27.8
28...	11	20.4	11	20.4
29...	18	33.3	13	24.1
30...	16	29.4	16	29.6
31...	17	31.5	14	25.9
November 1...	14	25.9	11	20.4
2...	10	18.5	13	24.1
3...	15	27.8	13	24.1
4...	17	31.5	14	25.9
5...	16	29.4	11	20.4
6...	12	22.2	15	27.8
7...	20	34.5	15	25.4
8...	13	22.4	12	20.3
9...	13	22.4	11	18.6
10...	16	27.6	13	22.0
11...	19	31.7	18	29.5
12...	21	35.0	15	24.6

TABLE XI (*Continued*).

Date.	Pen A.		Pen B.	
	Daily egg production.	No. of eggs per 100 birds per day.	Daily egg production.	No. of eggs per 100 birds per day.
November 13...	17	28.3	15	24.6
14...	20	33.3	16	26.2
15...	18	30.0	15	24.6
16...	20	33.3	18	29.5
17...	24	40.0	16	26.2
18...	19	31.7	15	24.6
19...	15	25.0	22	36.0
20...	23	38.3	14	22.9
21...	17	28.3	17	27.9
22...	24	40.0	20	32.8
23...	22	36.7	15	24.6
24...	19	31.7	21	34.4
25...	17	28.3	17	27.9
26...	19	31.7	16	26.2
27...	18	30.0	18	29.5
28...	22	36.7	17	27.9
29...	17	28.3	13	21.3
30...	16	26.7	18	29.5
December 1...	21	35.0	18	29.5
2...	16	26.7	18	29.5
3...	21	35.0	16	26.2
4...	16	26.7	18	29.5
5...	20	33.3	20	32.8
6...	12	20.0	14	22.9
7...	19	31.7	17	27.9
8...	19	31.7	20	32.8
9...	17	28.3	19	31.1
10...	20	33.3	17	27.9
11...	21	35.0	19	31.1
12...	17	28.3	21	34.4
13...	20	33.3	17	27.9
14...	14	23.3	19	31.3
15...	21	35.0	23	37.7
16...	19	31.7	17	27.9
17...	17	28.3	21	34.4
18...	16	26.7	19	31.1
19...	20	33.3	21	34.4
20...	21	35.0	19	31.1
21...	16	26.7	18	29.5
22...	24	40.0	22	36.1
23...	20	33.3	21	34.4
24...	21	35.0	16	26.2
25...	22	36.7	27	44.3
26...	18	30.0	22	36.1
27...	19	31.7	19	31.1
28...	19	31.7	24	39.3
29...	19	31.7	22	36.1
30...	21	35.0	23	37.7
31...	20	33.3	21	34.4
January 1...	22	36.7	23	37.7
2...	18	30.0	24	39.3
3...	22	36.7	19	31.1
4...	24	40.0	22	36.1
5...	18	30.0	20	32.8
6...	24	40.0	29	47.5

TABLE XI (*Continued*).

Date.	Pen A.		Pen B.	
	Daily egg production.	No. of eggs per 100 birds per day.	Daily egg production.	No. of eggs per 100 birds per day.
January 7...	24	40.0	17	27.9
8...	23	38.3	26	42.6
9...	21	35.0	20	32.8
10...	27	45.0	22	36.1
11...	18	30.0	20	32.8
12...	24	40.0	26	42.6
13...	19	31.7	17	27.9
14...	22	36.7	20	32.8
15...	18	30.0	20	32.8
16...	18	30.0	21	34.4
17...	15	25.0	20	32.8
18...	20	33.3	20	32.8

TABLE XII.

Number of eggs produced per pen per period.

Period.	Pen *A*.	Pen *B*.
1	366	344
2	102	93
3	106	91
4	131	110
5	144	119
6	128	120
7	125	121
8	128	132
9	130	138
10	143	151
11	146	154
12	155	154
13	136	144
Totals	1940	1871

These results in respect of egg production are striking, when taken in connection with the results on food consumption. We note:

1. The "free-choice" and mass-fed pens produced substantially the same number of eggs during the experiment, there being a slight excess, amounting to roughly 4 per cent., in favor of the free-choice birds.

2. Both pens gave a very good winter egg production, amounting to an average of over 30 eggs per bird with nearly a month and a half (January 19 to March 1) yet to go at the close of the experiment in order to complete the normal winter period (6). This fact is of importance as showing the general normality of the birds and their egg production under the experimental conditions.

3. During the first 7 periods the Pen A birds laid slightly more than the Pen B birds, while during the last 6 periods the relation was reversed, the differences in both cases being insignificant statistically.

Taking totals it appears that for each egg produced there was consumed, on the average, the nutrients indicated in Table XIII.

TABLE XIII.

Nutrients consumed by the flock per one egg produced.

Nutrient.	Pen A.	Pen B.	Per cent. increase Pen B over Pen A.
Protein	42.6 grams	66.4 grams	+55.8
Carbohydrates	228.2 "	227.1 "	− .5*
Fat	13.4 "	21.0 "	+56.4
Calories	1235.0 "	1398.4 "	+13.2

From this table it appears that the mass-fed birds consumed approximately 56 per cent. more of protein and of fat per egg produced than did the "free-choice" birds. The same amount of carbohydrate was consumed per egg in both cases. The energy intake associated with the production of one egg was 13.2 per cent. greater in Pen B than in Pen A.

It is apparent that the whole machinery of the domestic fowl is run much more economically in respect of energy and material intake when the birds are allowed freely to choose their ration from among the common poultry feeds, than under as good a system of mass-controlled feeding as has yet been devised. Whether this difference is specific for the reproductive portion of the machine we shall consider in the next section.

Body weight.

The birds used in this experiment, as has already been pointed out, were pullets. When they were brought in from the range and put in the laying house for the experiment their growth in body was not completed, though they were, as the egg records show, reproductively mature. They were weighed at monthly intervals throughout the experiment, with results shown in Table XIV.

It is at once clear from this table that:

1. The birds were properly selected for the experiment. At the first weighing the difference in mean body weight between the experimental and the control birds amounted to only .08 kg., an obviously insignificant difference.

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* Decrease.

TABLE XIV.

Average weight in kilograms of birds on days weighed.

Date.	Pen *A*.	Pen *B*.
Sept. 20	2.01	1.94
Oct. 27	2.39	2.27
Nov. 24	2.45	2.34
Dec. 22	2.52	2.44
Jan. 19	2.54	2.52

2. At the end of the experiment (actually 12 days before the final end) the two groups were only .02 kg. apart in mean body weight. In other words, in spite of the 30 per cent. lower intake of protein and fat on the part of the Pen A birds they were able to maintain equality of body weight with the Pen B birds, just as they have been shown above to have maintained equality of egg production. This can only mean markedly superior physiological utilization of the ration in the former case as compared with the latter.

Some general observations on body weight in relation to exercise are of interest. The birds in Pen B exercised more extensively than did those in Pen A because they found it absolutely imperative to do so, else they needs must forego the part of their daily meal made up by the scratch food. The birds in Pen A were by no means idle. During the day they would be found at almost any time, scratching and searching diligently in the litter as though they felt that perhaps there was a chance of finding a stray kernel of grain. The two hens that made the greatest gains were in Pen B where they had to scratch for their corn, wheat, and oats. Number 284 weighed 2.88 kgs. at the beginning of the experiment and 3.90 kgs. at the close, thus registering a gain of 1.02 kgs. She was the heaviest bird in the two lots. Number 320 of Pen A weighed 2.88 kgs. when put into the pen, and 3.72 kgs. at the close of the experiment, making a gain of .84 kg. The greatest gain made by any one bird was made by No. 298, a Barred Plymouth Rock pullet of Pen B, which weighed 2.25 kgs. at the beginning of the experiment (September 21), and 3.70 kgs. at the close of the experiment (January 19), registering a gain of 1.45 kgs. or .4 kg. per month. While the individual cases just mentioned are interesting they would be of little value as evidence for the entire flock in each pen, but the gains made by the flocks bear the same relation one to another. When put in the house the birds in Pen A weighed 110.81 kgs., and at the last weighing, after having lost one bird by death, the flock weighed 152.25 kgs., which is a gain of 41.44

kgs. The birds in Pen B weighed 104.49 kgs. when put in, and on January 19 at the last weighing they weighed 153.52 kgs, which is a gain of 49.03. Thus it would seem that the theory that birds will take on excessive weight if fed by the hopper method is not substantiated. Jackson got the same result, and has the following to say regarding the matter (*loc. cit.*, pp. 230–231). "It was feared that under the conditions of exclusive hopper feeding and close confinement, the hens would suffer from insufficient exercise. It is not possible to say what might have been the result of more liberty or a different system of feeding, but . . . there is no reason to think that the health of any was particularly affected by the conditions."

From the data as to total gain in weight and total nutrient consumption Table XV has been computed.

TABLE XV.

Nutrients consumed in association with the production of 55 grams gain in body weight by the flock.

Nutrient.	Pen A.	Pen B.	Per cent. increase pen B over pen A.
Protein	109.5 grams	139.1 grams	27.0
Carbohydrate	587.4 "	476.9 "	−18.8*
Fat	34.7 "	44.1 "	27.1
Calories	3180.0 "	2935.7 "	− 7.7*

We see here, just as in the case of egg production, that the free-choice birds made a much more economical utilization of their ration than did the control birds. For substantially the same gain in body weight the former group consumed 27 per cent. less protein and fat. The energy intake was, however, 7.7 per cent. more in Pen A than in Pen B. This arose from the carbohydrate relation. While the percentage difference between the two groups in respect of carbohydrate consumption was not large the absolute amount consumed is so large in proportion to protein and fat as to dominate the calory percentage.

The average weight of pullet eggs of the strain of birds on the Maine Station plant has been shown by Pearl and Surface (7) to be 55.264 gr. per egg. We have, therefore, taken 55 grams as the unit of the body weight table (Table XV). If we then compare the nutrient consumption associated with the production of the same amount (mass) of material as (*a*) egg and (*b*) body tissue, by the same birds at the same time, it is seen that, in general, something over twice as great average nutritive intake appears upon the unit of body-weight base, as upon the unit of egg-production base. It must, however, be remembered that the average of both Tables XIII and XV are inclusive. Egg production and growth in body-weight were both going on at the same time in the birds, while XIII and XV in a sense assume that all of the food intake went to a particular function only. The really only valid use of the figures of Tables XIII and XV, which can be made, is for comparing of the Pen A with the Pen B birds.

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* Decrease.

The nutrient intake of a laying pullet is used for the following purposes:

a. The maintenance and operation of the whole physiological machine as a going concern.
b. Growth (increase in body weight).
c. Production of eggs.
  1. Material and energy content of eggs themselves.
  2. Energy required to operate the egg-producing machinery.

Of these items *c*, 1 (the material and energy content of the produced eggs) can alone be separately determined. Using Atwater and Bryant's factors we have for the total eggs laid by the two groups the results shown in Table XVI.

TABLE XVI.

Nutrients in all eggs produced during experiment.

Nutrient.	Pen *A*.	Pen *B*.
Protein	14.045 kg.	13.545 kg.
Fat	9.971 "	9.616 "
Calories	150,315	144,963

It is at once apparent, that so far as actual output of material and energy in the form of eggs is concerned there is no essential difference in the two groups. Hence it must follow that the Pen B birds made a much less economical use of their nutritive intake in respect of the other physiological processes (*a*, *b*, and *c* 2 of the classification above) than did the birds of Pen A. If we subtract the figures of Table XV from those of Tables VI and VII we get the results shown in Table XVII.

TABLE XVII.

Total nutrients consumed for maintenance and operation of all physiological processes except output of eggs (net nutrient content of eggs produced deducted from gross total intake).

Nutrient.	Pen A.	Pen B.	Percentage increase of pen A over pen B.
Protein..............	68.603 kg.	110.590 kg.	+61.2*
Carbohydrate.........	442.615 "	424.892 "	− 4.0*
Fat..................	16.097 "	29.712 "	+84.6
Calories.............	2,245,696	2,471,798	+10.1

From this table it is seen how enormously more efficiently the Pen A birds utilized their nutrient intake for the maintenance of their general physiological economy than did those of Pen B. The case is like that of an inefficiently stoked as compared with a well stoked steam boiler fire. One can get the same energy output from the plant with a poor fireman as with a good, both using the same kind of coal, but only at the expense of a greatly increased coal consumption.

Discussion.

The broad, outstanding result of this work is that a flock of 60 pullets, when given entire freedom as to choice of their ration from among the common whole and milled grains, produced somewhat more eggs and made substantially the same gain in body weight as an equal group of birds fed according to one of the best standard methods of poultry feeding, and accomplished these results with a net nutrient consumption of 33 per cent. less protein, 34 per cent. less fat, and 8 per cent. smaller total energy intake. This is a remarkable result, and may have highly important consequences for the economic management of domestic poultry. It should be emphasized that save in respect of freedom of choice of food, both flocks were managed in exactly the same manner. They had equal floor space in identically the same pens, the same amount of water, and in every other possible particular except the feeding were treated in the same way. There was nothing peculiar or troublesome in the management. The management of large commercial flocks of poultry in the same way that our Pen A was treated is entirely practical and feasible, and will indeed involve far smaller labor costs than any other system of management yet devised. Whether it will be economically desirable to do this depends mainly on the relative costs of the different whole and milled grain products at the time. In this question we are not now interested.

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* Decrease.

A word of caution should be noted by anyone thinking to repeat these experiments. In order to get clear-cut results with poultry in an experiment of this kind many years' experience has convinced the senior author that a degree of knowledge of the genetic or hereditary characteristics of the individual birds is requisite, particulary in respect of fecundity, which is not likely to be possessed by any poultry farmer, and by but extremely few experimental plants. By a haphazard selection of experimental and control birds in respect of their genetic fecundity characters one might easily get results which would be widely divergent from those here reported. In this experiment the genetic constitution of every bird in respect of fecundity was thoroughly known, on the basis of individual pedigrees extending over many generations. The birds were so selected as to make the two pens genetically identical in respect of this important character. We should consider no experiment an adequate repetition of this one, whatever its results, in which it could not be demonstrated that this genetic identity between experimental and control birds was maintained. Most of the experimental results on feeding poultry, and other live stock for that matter, are, in a critical scientific sense, of very doubtful validity, just because the genetic factor always involved is so completely neglected. The senior author has come to feel that really before almost any sort of animal experimentation is undertaken it is essential that a stock be prepared of known and stable genetic constitution, by the necessary amount of controlled pedigree breeding, often requiring years of time. The debonair fashion in which physiological experimenters, using "physiological" in the broadest sense, draw conclusions from experiments often most elaborate in character, but made upon animals purchased from the animal dealer, about the innate individual constitutional peculiarities and differences of which the experimenter can possibly know nothing, gives one to wonder afresh at the naïve simplicity of the human animal.

One other point: Most of the experiments hitherto carried out with poultry upon the "cafeteria" mode of feeding have been done by putting single individual birds in pens or coops alone, each by itself. From extensive observations on the normal psychology of the domestic fowl, we doubt the significance of results got in this way. The hen is a gregarious creature, and is rather profoundly and apparently permanently upset by being forced to live alone. One does not look for normal results in respect of such subtle psychological matters as appetite under these conditions. Furthermore if the experiments are ever to have any practical meaning for poultry husbandry they must duplicate in some degree at least the practical conditions under which poultry is kept commercially.

SUMMARY.

The experiment here reported was designed to test what the effect would be upon egg production and gain in body weight of permitting domestic fowls to choose freely for themselves what kinds and amounts of food materials they would eat. An experimental flock of 60 birds was allowed free access to some 12 different common food materials presented to them at all times in hoppers, while a control flock, selected to be as nearly as possible of identical genetic constitution in respect of fecundity, was fed according to the system normally used at the Maine Agricultural Experiment Station, and found by years of experience there and in the hands of practical poultry men to give excellent results.

The chief results were:

1. The free-choice birds ate more of whole grain and less of milled products than the mass-fed birds.
2. The free-choice birds consumed 33.4 per cent. less protein, 33.7 per cent. less fat, and had a total energy intake in calories 8.4 per cent. smaller than the corresponding consumption (including wastage) of the mass-fed birds. The consumption of carbohydrate was somewhat, but not greatly higher in the free-choice birds.
3. With food consumption differences indicated above, the egg production, which in absolute amount was excellent, and the gains in body weight, were substantially identical in the two groups.
4. The conclusion appears justified that when fowls are allowed complete freedom of choice in the kind and amount of the food they ate, they make a better physiological utilization of their rations than under a system of man-controlled mass feeding, however excellent theoretically the system may be.

LITERATURE.

1. PEARL R.
1913. The biology of poultry keeping. Me. Agr. Expt. Stat. Ann. Rept. for 1913, pp. 101–120.

2. Evvard, J. M.
1915. Proc. Iowa Acad. Sci. XXII.
3. Jackson, H. W.
1911–1912. Ann. Rept. Penn. State Coll., pp. 228–41.
4. Kempster, H. L.
1917. Jour. Amer. Assn. Inst. and Inv. Poult. Husb., pp. 26–32.
5. Pearl, R.
1920. The nation's food, Philadelphia.
6. Pearl, R.
1912. Jour. Exp. Zool., XIII, pp. 153–268.
7. Pearl R. and Surface, F. M.
1914. A biometrical study of egg production in the domestic fowl. III. Variation and correlation in the physical characters of the egg. U. S. Dept. Agr. Bur. Anim. Ind. Bull. 110, part III.

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I. The Mathematics of Biometry.[1]

By Lowell J. Reed, Johns Hopkins University.

I think we are all agreed that the program for this meeting of the Mathematical Association of America is as important as any that the Association has ever discussed. Any science that fails to ally itself with the other sciences must of necessity have a narrow development, and this is perhaps more true of mathematics, due to its wide application, than of any of the other sciences. It is to be hoped that the Program Committee will carry out its own suggestion that at some later meeting we consider the converse of the present question, that is, the contribution of other sciences to the development of mathematics.

My own part in the present program is the discussion of the mathematics of biometry, and instead of presenting illustrations of the application of mathematics to this branch of biology I am going to outline what I consider to be the proper mathematical training for work in biometry. I wish first to call attention to the development of the present method of teaching mathematics. We have in the field of mathematics a number of branches that have been developed mainly as tools for the solution of practical problems. Thus, trigonometry has been developed mainly for the solution of problems in surveying; probability has been developed for games of chance. Now whenever this has been the case the teaching of the subject has been concentrated on this one particular application, to the exclusion of all others. In recent years the increasing use of mathematical methods in such sciences as chemistry, biology, etc., has led to an effort to broaden the teaching of mathematics by introducing new applications. The result has been a new group of textbooks under such titles as "Calculus for Chemical Students," "Mathematics for Agricultural Students," etc. All of these texts seem to me to miss the point in that they imply that calculus for chemistry students is distinct from calculus for other groups. This I do not believe to be the case. If we consider the mathematical needs of a student in any one of the sciences we find them about as follows: First, he needs a foundation in algebra, trigonometry, analytic geometry, and calculus; and secondly, he needs to be trained to take a problem in his particular field and translate it into mathematical language. The latter need is the greater of the two, and it is the one that it is the more difficult to satisfy.

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[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 10. Read before the Mathematical Association of America, January 1, 1920.)

I now wish to present what seems to me to be the proper mathematical training for work in biometry; although the following courses are outlined with the needs of a student of biology in mind, I feel sure that they are the proper ones for students in any branch of science. The first year courses in mathematics should cover the three subjects, algebra, trigonometry, and analytic geometry. The course in algebra should be fully as complete as the one at present given and may occupy the first half of the year. Emphasis should be laid on problem solution with the idea of satisfying the second of the needs listed above. The course in trigonometry should give to the students a knowledge of the trigonometric functions, showing their relations to each other and to the right-angled triangle; it should develop the relations that exist between the function of the angle and the sides of the general triangle, but it should not contain the usual extensive drill in the application of these formulas to the solution of triangles. The use of the Law of Sines and Law of Cosines in mechanics is fully as important as their use in solving triangles and the application is not obscured by a long computation, but one very seldom sees such applications in a textbook on trigonometry. The course in analytic geometry should give the methods of the subject with a fairly complete discussion of the straight line and circle, and a brief discussion of conics. In this course again there is an excellent opportunity to train students in the methods of expressing ideas in mathematical notation. To the first year's work might well be added a short section on computation so that the students could become acquainted with the use of logarithms, and if possible learn to use some of the various calculating machines.

The second year's work should be divided into two parts. The first half year should be devoted to a course giving the elements of calculus, emphasizing principles and methods rather than the tricks of differentiation and integration. In the second half of the year the students should be divided into groups according to their interests, one group for those majoring in engineering, another for those majoring in the natural sciences, etc., the divisions formed being adjusted to suit the needs of the institution in question. Then the students in each group should be given work applying the preceding mathematics course to their respective fields. The students majoring in different natural sciences should not in my opinion be further separated into distinct groups as they are all concerned with the same fundamental problem, that is, the analysis of observed data, and the course given to this group of students should train them in modern statistical methods of analysis. First, the subject of probability should be presented from its theoretical side. This should include the fundamental definitions and a discussion of probability theory leading to the normal curve. The meaning of the constants of the normal curve and the notion of probable error should be introduced at this point. The subject of probability should be followed by a discussion of the principle of moments, illustrations being drawn from mechanics. The treatment of this subject should lead naturally into a discussion of frequency distributions and correlation. The relationship of the first, second, and third moments to the mean, standard deviation, and the skewness of frequency distribution, should be clearly brought out, mechanical illustrations being freely used. The correlation coefficient should be developed as a product moment with the mean of the variables taken as an origin. Following this the students should be taught the theory of curve fitting by the method of moments and this theory should be illustrated by examples in the derivation of empirical formulas. The method of fitting curves by least squares need not be presented as it is not so widely applicable as the method of moments. The student should also have impressed upon him the importance of the idea of probable error and should be trained to work out the probable error of all such constants as mean, standard deviation, coefficient of correlation, etc.

The course just outlined would furnish a student in biometry with the proper mathematical foundation for his work; his mathematical needs are similar to those of the student of any natural science. One of the most common quotations among biometricians is that of Sir Francis Galton: "Until the phenomena of any branch of knowledge have been submitted to measurement and number it cannot assume the status and dignity of a science," and as we see more and more of the leading scientists taking this point of view it impresses upon us the growing importance of mathematics. The methods of teaching mathematics should develop accordingly; and if this present meeting succeeds in stimulating the teaching of mathematics to live up to its increased importance, it will indeed have been a success.

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THE NATURAL HISTORY OF TYPHOID FEVER IN BALTIMORE, 1851-1919 *

By WILLIAM TRAVIS HOWARD, JR.

INTRODUCTION

In discussions on the course of typhoid fever in any community over a considerable number of years, it has been the custom to assume that there have been, during the periods under study, at least four constants: First, that, on the whole, the population has remained constant in regard to susceptibility to the disease when exposed; second, that both the invasive and the killing capacities of the causal agent have been, on the average, the same; third, that the honesty and the diagnostic acumen of physicians, as well as the available methods of diagnosis and the clinical course of the disease have all remained relatively unchanged; and fourth, that the results of the medicinal treatment and management of cases of the disease have undergone no important variations. As a matter of fact, in most communities at least, such an assumption is unwarranted, and Baltimore is no exception to the general rule that variations in all these factors have taken place between 1851 and 1919, the period covered in the present study. [276]

The population has been under a continual change; one not attributable to loss by death and replacement by birth. New racial elements have entered, and a larger proportion of those born now reach the typhoid age than in former years. Just before our period opens, and running well into its first twenty-five years, there was a large immigration of Irish and of Germans which raised these stocks to a much greater proportion of the population than had obtained previously. Such interesting race stocks as the Russian and Polish Jew, the

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* Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 15.

[276] Czech, the Lithuanian, the Polish, and the South Italian have all joined us since the date when the typhoid rate began to decline. Representatives of other races in smaller numbers have come in more recent times. The numerical proportion of negroes to whites in the population has remained relatively unchanged at one-sixth. Have the immigrants added to or subtracted from the proportion of our typhoid susceptibles? The important questions of the transmission of either typhoid susceptibility or non-susceptibility to the progeny of those who survive attacks of the disease and of the value to be set upon the elimination of typhoid susceptibles through death from this or from other diseases are wholly ignored.

It has been the habit to assume, at least tacitly, that each city has its own typhoid bacillus strain of unvarying infective and lethal capacities. This is far from true, for additions of new races, or strains, of typhoid bacilli are being brought in continually, often from distant parts of the world, and are being diffused; again, old ones are perhaps changing their characteristics or even dying out. It is by no means certain that case fatality rate among typhoid fever cases is in any place, whether it is an open or a closed community, constant over any considerable period of time. In fact, there is much evidence that the contrary is true.

Nor is it possible to estimate accurately the influence of diagnosis, or of fashion in diagnosis, upon typhoid death rates. Bilious fever and malarial fever as causes of death died hard. Pathological anatomists are familiar with the wide margin of error in the diagnosis of typhoid fever. They have found only too often the lesions of this disease and the typhoid bacillus in bodies coming to the autopsy table with diagnoses of pneumonia, empyema, meningitis, tuberculosis (especially general miliary), appendicitis, acute endocarditis, influenza, uræmia, etc., and perhaps the reverse has been experienced as often. Intelligent health officials are well acquainted with these sources of error. In 1919, physicians in Baltimore withdrew the diagnosis of typhoid fever, reported by them to the Health Department, in 42 cases, the most common diagnosis substituted being pneumonia, empyema, influenza, intestinal intoxication, and tuberculosis. Some of these patients had been sent to hospitals by practitioners as cases of typhoid fever. [276] On the other hand, in a considerable number of instances, cases of typhoid fever were first reported as pneumonia or as influenza. Again, it is only comparatively recently that para-typhoid fever has been elevated to the position of a separate disease, and even now while para-typhoid and typhoid fevers, as reported cases, are classified separately in the Health Department records, deaths from the former are still charged to the latter in the international classification.

The question whether the larger experience given physicians during the former period of greater prevalence of typhoid fever did not counterbalance the influence of the more refined methods now available is at best an open one. Outside of exceptionally well organized hospitals, the blood culture method of the diagnosis of typhoid fever is relatively seldom used. Every public health administrator who has carefully studied the subject must be aware that, under the conditions [277] which must of necessity obtain in its application and interpretation, the Widal reaction is often a source of error. When a single test is relied upon, many cases of typhoid are excluded, and, in numerous instances, cases of other diseases are classed as typhoid. This is due in the main to three sources of error: the use in the diagnostic laboratories, year in and year out, of a single strain of *B. typhosus,* usually one that is highly sensitive to human serum; the fact that the degree of dilution of the serum is mere guess-work, depending not only upon the bacteriologist's judgment in color, but upon the relative amount of coloring matter in a given amount of the blood of the subject of the test, *i. e.,* his degree of anæmia or plethora; and the fact that in several acute affections, notably influenza, endocarditis, and miliary tuberculosis, which are most apt clinically to be confused with typhoid fever and to suggest to the physician the advisability of appealing to the Widal reaction, the blood serum often acquires the property, even in considerable dilution, of agglutinating *B. typhosus.* These considerable sources of error, while known to many public health bacteriologists, are not so well appreciated by many practising physicians and public health administrators. In a large proportion of the 42 cases of withdrawn diagnosis of

[277] typhoid fever above alluded to, the attending physicians were misled by positive Widal reactions. Notwithstanding these drawbacks, the Widal reaction, as applied under the relatively unfavorable conditions inherent to public health laboratory service, is of great service to both practising physician and public health administrator and has undoubtedly promoted accuracy of diagnosis. The laboratory of the Health Department has perhaps been the most effective agent in rectifying the old confusion of malaria with typhoid fever in Baltimore. That some physicians have a habit of burying their fatal cases of continued fever under the diagnosis of typhoid fever is well known in the Health Department. The affections sought to be covered under typhoid fever as a cloak are especially puerpural fever, surgical infections, and tuberculosis.

My impression, after four years experience and a study of the records of the Health Department, is that, on account of errors in diagnosis and deliberate misstatements on death certificates, the number of registered deaths from typhoid in Baltimore are certainly below the actual number in the earlier period and probably above it in the later period covered by this study. This is, however, to some degree, balanced by my inclusion of deaths registered as due to typho-malaria with deaths from typhoid fever.

Many facts lead to the conclusion that under the influence of the great improvement in living conditions, using the term in the broad sense to cover improvements in hospitals and in nursing, the substitution of bathing and sponging and of the expectant for the drastic treatment of typhoid fever that has marked the last thirty years, the physician has been able, on the whole, greatly to improve the typhoid patient's chances of recovery.

Though the knowledge necessary for the proper evaluation of these and perhaps other important factors of error is lacking, attention is directed to them, for, so far as I am aware, in discussions such as the present one, they have been left out of consideration, and it has been very generally assumed as true that the forces of morbidity and mortality of *B. typhosus,* on the one hand, and the material upon which they act, on the other, have remained constant. With the exception of some figures on the white and colored and on the two sexes, there are now available no satisfactory data for a study of the genetic relationships of typhoid fever in Baltimore. This study covers, then, for the most part, a consideration of some of the environmental relationships of typhoid fever in the population of Baltimore from 1851 to 1919, inclusive. [277]

The morbidity and mortality figures, the case histories, and much of the general data were obtained from records of the Health Department, and for these I am under deep obligation to Dr. C. Hampson Jones, Commissioner of Health, Dr. John F. Hogan, Assistant Commissioner of Health, Dr. William Royal Stokes, Bacteriologist, Dr. Marion B. Hopkins, Chemist, Mr. Moore and Miss Betz of the Division of Statistics, and Mrs. Bertha M. Muller. To the Health Wardens and to the Nursing Staff I am indebted for histories of reported cases.

The population figures used in obtaining morbidity and mortality rates are not those of the Bureau of the Census. They were calculated by Mr. John Rice Minor, at the suggestion of Dr. Raymond Pearl, by fitting a logarithmic curve to the population figures of Baltimore, as given by the Bureau of the Census for the censual years. These population figures, representing more accurately, it is believed, than those given by the Bureau of the Census, the populations of both censual and intercensual years, are used constantly in this laboratory in work on the morbidity and mortality data of Baltimore. As the population figures are somewhat higher than those of the Bureau of the Census, the rates are somewhat lower, particularly in the latter years, than those of the Health Department and of the Bureau of the Census.

THE EARLY HISTORY OF THE COURSE OF TYPHOID FEVER IN BALTIMORE

Typhoid fever was not recognized officially by the Health Department as a cause of death until 1851. It was probably hidden under the terms bilious, malarial, typhus, nervous, and gastric fevers, and diarrhœa and dysentery in the early years of the Town and City, and during this period bilious, typhus, and malarial fevers were all accredited with a considerable number of deaths. Nervous fever appears as a cause of death in from 1 to 9 cases in each year from 1812 to 1824.

[277] Gastric fever was first given as a cause of death in 1837, and the number of deaths from this affection gradually increased from 3 in 1837 to 30 in 1850, after which this cause declined. Bilious fever was given as a prominent cause of death from 1812, the earliest date to which I can refer, with 99 deaths. It declined to 53 deaths in 1852. It is credited, however, with 72 deaths in 1853. By 1875 it had declined to 1 death and entirely disappeared after 1885.

It is interesting that Thomas H. Buckler, William Power, William T. Howard, Frank Donaldson, Christopher Johnson, [278] J. W. Houck, Charles Frick, and others, including medical students at the Almshouse, were familiar with the clinical course and anatomical lesions of typhoid fever in the early 40's. Buckler and Power were visiting physicians, and the others mentioned were resident physicians, to the Almshouse and its Infirmary as early as 1843. J. K. Mitchell and other professors of medicine clearly recognized the disease in their lectures and writings in the 40's. It was, however, not until after William Jenner's paper, read on December 11, 1849, before the Royal Medical and Chirurgical Society of London, that the Health Department included typhoid fever as a cause of death. This is the more curious because William Power, generally recognized as the leading clinician of his time in Baltimore, was a pupil of Louis from 1835 to 1838, and was Professor of the Practice of Medicine at the University of Maryland in 1846. Bartlett, who preceded him in the Chair of Practice, was thoroughly familiar with not only the work of Louis, but that of Shattuck in London, Stewart in Glasgow, in 1839 and 1840, and Gerhart in Philadelphia, in 1837, and published his epochal book on continued fevers in 1842. In 1851, Thomas H. Buckler recorded that a very grave form of typhoid fever prevailed among the inmates of the City Almshouse in 1846, 1847, 1848, 1849; that in the latter three years the wards were never free from the fever; and that the resident physicians and students in the Infirmary rarely escaped the disease. Buckler did not mention the degree of prevalence of typhoid fever in the City proper, but he stated that Dr. Frank Donaldson had demonstrated the intestinal lesions of typhoid fever in an immigrant at the Quarantine

Hospital in 1847. In the same year, two immigrants with [278] typhoid fever were sent from an immigrant ship to the Almshouse Infirmary. The reports of the Quarantine Officer about this date not infrequently mention cases of typhoid fever.

In the tabulation of the medical and surgical cases treated in the infirmary of the Almshouse in 1828-1829, there were recorded 58 cases, with 13 deaths, of *febris typhoides.* The term *typhoid fever* was first used in the annual report of the Almshouse in 1833, when there were 13 cases and 3 deaths. The annual reports of the attending physicians recorded cases of typhoid fever every year from 1833 to 1849, inclusive, with the exception of 1837. In these seventeen years, there were 359 cases and 106 deaths, with a case fatality rate of 29.53 per cent.

The earliest date of the appearance of the disease in Baltimore is unknown. It was perhaps introduced early and became increasingly prevalent during the enormous growth of the City between 1790 and 1830. It is practically certain that a great many cases of this fever reached Baltimore in the early 40's and early 50's among the great crowds of Irish immigrants who brought typhus and smallpox as well. It is likely that typhoid fever gained a hold in the City at an early date and spread in the usual ways, but especially by pollution of springs and wells and milk and by the mediation of flies, favored by the constant overflowing of the crude privies and privy wells. It is probable that in its earlier days the Lake Roland water supply was not seriously contaminated. With the increase of the suburban population on this watershed, and later on the Gunpowder watershed, these water supplies became very seriously contaminated. In the meantime, the city springs and wells became more and more polluted, the privies became more dangerous, and milk became a very grave menace.

GENERAL SANITARY CONDITIONS IN 1850

In regard to sewers and the disposal of night-soil from privies and privy wells, the foul pictures given in the report of Wynne in 1849 and the reports of the Health Department from this date until a relatively recent period could only be duplicated in the London of the beginning of the 19th cen-

[278] tury. Harford Run and Jones' Falls to the east and Chatsworth Run and Schroeder's Run to the west duplicated in their foulness the old Fleet and Holborn sewers of London. The Basin into which these filth-streams emptied was perhaps worse than the Thames. It was so foul, that, according to Thomas H. Buckler, as late as 1875 its rotten egg odor commonly reached "Evergreen," his place just north of the present North Avenue, and with a favorable south wind it was clearly detected at Towson. The privies and privy wells in yards and even in the cellars of some of the best houses were constantly overflowing into cellars, courts, lanes, alleys, and streets. The low-lying parts of the City, at Fells Point, along the outlet of Jones' Falls, in the section bordering on the Basin, and the southwest section near Spring Gardens, were in a constant state of nuisance, and large portions of these districts were subject to flooding after very heavy rains. The higher section, with well marked hills and valleys, was far cleaner.

The scavengering and street cleaning were under the control of the Health Department. For this purpose the City was divided into five districts, the cleaning of each of which was let out to separate contractors. The contractor received in payment the street refuse, which he sold as manure, and about one thousand dollars in money. The streets received attention in proportion to their tendency to collect filth, but never more than twice a week, more usually once in two weeks. The numerous narrow alleys and courts, inhabited by the poor, and the depositories of garbage and worse, received little attention and were generally in a filthy condition. The most effective scavengering in these districts was done by swine which were allowed, although it was against the law, to roam through them at their pleasure. The copious showers which were frequent especially in summer, brought about the best results in street cleaning for the City. The streets were often of defective grade and in general roughly paved with cobblestones. There were storm-water gutters on each side, but as sewers were almost non-existent, there being only about two miles of storm-water sewers in the whole City, they led the water through and over streets directly into the Basin or into the nearest stream. With this storm-water went kitchen, bath, and laundry water and much garbage, as well as street [278] manure and material from overflowing privies and cesspools. Between rains these streams were sluggish and in hot weather emitted offensive odors. The garbage and street sweepings removed by the contractors were dumped on convenient vacant [279] lots at the borders of the City until disposed of.

The night-soil from privies and cesspools was removed by licensed night-soil contractors and sold directly to farmers or manufactured into poudrette. At this time, a portion at least of the night-soil was deposited on the garbage and street manure dumps, and much of its was shipped out of the City on scows.

The nuisances from overflowing privies were a constant source of annoyance and danger to health.

In 1849, not more than one-half, and perhaps not more than one-third, of the population drank water from the public water supply derived from Jones' Falls; the remainder derived its water supply from springs and public and private wells. Many, if not most, of these last must have become grossly polluted by this time from the supersaturation of the subsoil from the numerous cesspools or from surface pollution from privies and overflowing cesspools. It is certain that the public water supply was at this date to some degree polluted from the settlements that grew up around the numerous mills dotting the course of Jones' Falls above the intake.

The better-to-do inhabitants, including the skilled laborers, lived in well built, single-family, brick houses. A considerable portion of the population, consisting mainly of recent immigrants and negroes, lived crowded together in small houses in narrow, dirty, ill-paved, and poorly drained alleys, with an inadequate water supply.

SEWERAGE

As previously indicated, at the time at which our period opens, there were no sanitary sewers in the proper sense of the term, and the underground drainage for storm water was very meagre. According to Wynne, in 1849, the aggregate of all the sewers did not exceed two miles in length. Previous

[279] to 1862, the few existing storm-water sewers had been put in in a haphazard way.

The first serious consideration of the sewerage question on the part of the City government was in 1859, when a Sewerage Commission was appointed, consisting of Henry Tyson, John Dukehart, and J. Mowton Sanders. In their report rendered in 1862, commonly called the Tyson Report, the Commissioners recommended the establishment of eight sewerage districts, in which sewers were to be built to carry off storm-water and household waste waters, exclusive of human excreta, more readily along the natural drainage lines. Jones' Falls, in which there was an awkward bend to the westward in the neighborhood of Saratoga and Lexington Streets, was to be straightened, and it was proposed that an intercepting storm-water sewer, running parallel to this stream, be built. Some of the recommendations of this Commission, including the straightening of Jones' Falls and the supporting of its walls with masonry, were carried out during the next few years, but no systematic record was kept of the dates and courses of many of the new sewers.

At the invitation of the city government, C. H. Latrobe, C. E., in 1881, made an extensive report on the sewerage question. Mr. Latrobe, recognizing that the subsoil had reached the limit of its capacity to care for human excreta by the cesspool and privy systems, recommended a plan for a dual sewerage system: one for surface water and rainfall, to discharge as before into the middle branch of the Patapsco (Spring Gardens), into the Basin, and into the Harbor; and the other for household waste and human excreta, to discharge partly into Middle River (the middle branch of the Patapsco) and partly into the lower harbor below Canton. Relatively very little was done in regard to the storm-water drains, and the recommendations for sanitary sewers were entirely passed over.

Between 1880 and 1890, the construction of storm-water sewers was continued in an irregular way, and Harford, Chatsworth, and Schroeder's Runs were covered over, but in the meantime, there had been a very considerable increase in the number of cesspools draining into the new and the old storm-water sewers which discharged into these runs, or Jones' Falls, [279] or directly into the Basin and into the middle branch of the Patapsco. Many of the sewers, including these runs, were improperly trapped and gave off offensive odors. The uncovered Jones' Falls was, to a very great degree, a combined storm-water and sanitary sewer carrying so much filth that it was constantly necessary to dredge its lower portion for the removal of sediments of organic matter.

A second Sewerage Commission, under the chairmanship of Mr. Mendes Cohen, engaged Messrs. Rudolph Hering and Samuel M. Gray as Consulting Engineers and Mr. Kenneth Allen as Assistant Engineer. After an exhaustive study, the Commission, in its report of 1896, recommended a dual sewerage system; the storm-water to be discharged as recommended before, and the domestic waste, including human excreta, to be disposed of by dilution into the deep waters of the Chesapeake Bay, well below the City. Of the two alternative methods proposed by the engineers, *i. e.*, chemical precipitation before such discharge and the use of filtration beds on land in Anne Arundel County, they objected to the former as unnecessary, and to the latter on account of its great expense.

The city government, fearing injury to the fishing industry, particularly on account of the danger of infecting oysters with typhoid bacilli, directed further investigation and a report on the best alternative plan. The Commission then suggested that the plan of land filtration be tried, but be restricted at first to the sewerage from the low level area, thus serving about one-third of the population. The whole matter was then dropped because the people refused to approve a bond issue to pay the cost of the work.

In 1905, the third Sewerage Commission was appointed. Messers. Hering, Gray, and Stearns served as a board of consulting engineers, and Mr. Calvin W. Hendrick was appointed Chief Engineer. Mr. Hendrick and his staff designed and superintended the building of a double system of sewers—surface water and sanitary—covering most of the built up sections of the City. The surface water sewers discharge the storm-water as before. The sanitary sewers carry domestic waste and human excreta to a point on Back River, to the

[289] southeast of the City, where the sewerage is purified and the effluent discharged into the Bay. The system was completed in 1915. Connections were started on a limited scale in the fall of 1911 and were completed in 1917 and 1918. It was estimated by the Chief Engineer that the household sewerage of about 15,000 houses had been discharging into the Basin and Harbor. There are now over 90,000 houses connected, and in most of the old twenty-four wards, the privy and the cesspool have been eliminated. Thus, the opportunities for fly-borne typhoid must have been greatly restricted.

Much of the sanitary sewerage of a considerable district in the northwest and southwest sections of the City still finds its way into Gwynn's Falls, a stream already polluted with sewerage before it reaches the city limits. As the population so served is still relatively small and the stream is large and rapid, the insanitary conditions are by no means a nuisance comparable to those which obtained in Jones' Falls, or Harford, Chatsworth, and Schroeder's Runs in former days. Jones' Falls and its tributaries as well still receive a certain but relatively small amount of sanitary drainage from outlying sections of the city. The same may be said in regard to Herring Run, as it runs through the northeastern part of the City. This stream is already seriously polluted from sources to the north of the City. The closely built up Highlandtown, bounding the eastern margin of the City, still retains to a large degree the old, primitive method of storm-water and sanitary sewerage disposal which obtained in the old City before the construction of the new sewerage systems.

WATER SUPPLY

From its foundation until 1808, Baltimore depended upon the springs and wells within the City for its water supply, and perhaps to a certain extent, at its borders, upon the several streams. As early as 1792, the legislature gave permission to an insurance company to organize the Baltimore Water Company, with the privilege of supplying water to private users. The Company, however, did not avail itself of this privilege. In 1800, the legislature authorized the Mayor and City Council to introduce water into the City. On account of various delays on the part of the city government, [280] on April 21, 1804, at a public meeting of citizens, a Committee, under the chairmanship of General Samuel Smith, and including John Eager Howard, was appointed to organize a stock company for this purpose. The Company was organized on May 24, 1804.

Jones' Falls having been chosen as the source of supply, a reservoir was constructed near what is now the southwest corner of Cathedral and Franklin Streets, and a wheel and pumps, located at the present site of the Calvert Street Station of the Northern Central Railroad Company, forced water from a common mill race on Jones' Falls, known as Keller's Dam which supplied Salisbury Mill, situated near the site of the old Belvedere Bridge, just east of the present Guilford Avenue Bridge. The main surface pipes were hemlock logs, with bores from 1-½ to 4 inches in diameter, joined by driving the spigot-shaped end of one log into the bell-shaped end of its mate. The joints were bound with wrought irons bands. The main valves were of cast iron with tapered spigot ends which were driven into the wooden mains, the valves being opened by lifting the valve plugs by means of hooks. The small surface pipes were cedar logs 6 inches in diameter with a 1 inch bore. The first iron pipes laid by the Water Company were imported from England about 1820. The Company supplied the city fire plugs and extended services to private dwellings as and when the revenue to be derived was sufficient to pay the cost and a profit. Service began in 1808. There were always comparatively large areas to which this water supply did not extend, or where it was not taken advantage of, and in which the water of springs and wells was used as in the earlier days.

Somewhat later, the Water Company erected a new pumping station near the old Belvedere Bridge and built an additional reservoir on the high ground at the northeast corner of the present Chase and Charles Streets and a third reservoir called the Mt. Royal Reservoir situated near the present Union Station of the Pennsylvania Railroad. This was supplied by natural flow with water from the mill-race of the Lanvale Cotton Company situated higher up on Jones' Falls.

[280] It will be noted that the Water Company did not undertake the construction of dams and large impounding reservoirs along the course of Jones' Falls, but obtained its water supply from the dams of industrial mills which dotted the course of the stream.

John Randel, Jr., in his report to the city government in 1836, recommended that the water be obtained from the Gunpowder River at Great Falls, that for this purpose a dam be built at Tyson's Mills and another across the Western Run, near York Pike, and that, with an aqueduct joining these two sources at Beaver Dam, water be led through the ridge of limestone rock and thence along the western slope of Jones' Falls Creek to a reservoir 300 feet above tide water near Baltimore.

According to Wynne, the service pipes to households, after 1820, were exclusively of lead. In 1849, he estimated that 5000 houses were supplied with hydrants. The City provided free hydrants in certain sections for the poor. At that time the average daily consumption of water was 500,000 to 1,000,000 gallons in the summer and much less in the winter. He describes the water as pure and soft and agreeable to the taste.

The City took over the Water Company in 1852, and after a sharp discussion as to whether the new water supply should be obtained from the Gunpowder River or from Jones' Falls, the contest was decided in favor of the latter. A dam was built at Relay House on the Northern Central Railroad, at an elevation of 220 feet above mean tide. From this, water was led by natural flow through an aqueduct four miles long to a high service reservoir in Hampden and thence by cast-iron pipes for distribution in the City and also to a low service reservoir at North Avenue and the Falls. This system was put into use in 1862. The impounding lake, now called Lake Roland, had an available capacity of 400,000,000 gallons.
[281] The Hampden Reservoir had a storage capacity of 50,000,000 gallons, and the present Mt. Royal Reservoir had a capacity of 30,000,000 gallons.

In 1870, the present Druid Lake in Druid Hill Park, with a storage capacity of 429,000,000 gallons, was completed.

Four years later the West High Service Reservoir in Druid [281] Hill Park, with an elevation of 350 feet above mean tide was finished. This reservoir was designed to serve the rapidly growing western and northwestern sections of the City.

In 1862, the consumption of water was not over 8,000,000 gallons of water a day, and it was estimated that the new supply would furnish not under 20,000,000 gallons a day. On account of a severe drought in 1872, the City faced a water famine, and it became evident that the Jones' Falls water supply would have to be reinforced. For this reason, in 1874, a dam was constructed at Meredith's Ford on the Gunpowder River, and 5,000,000 gallons of water a day were forced through a main over the dividing ridge between the Gunpowder River and Jones' Falls into the Channel of Roland Run, a tributary of Jones' Falls, above Lake Roland.

The City then set to work to obtain the larger part of its water supply from the Gunpowder River. In 1875, the following extensive works were begun: An impounding reservoir on the Gunpowder River at Lock Raven with a capacity of 510,000,000 gallons and with a dam 800 feet long and 30 feet high; a gate-house; a supply tunnel 7 miles long, leading to a receiving reservoir at Lake Montebello with a storage capacity of 500,000,000 gallons; and a conduit a mile long from this lake to a gate-house at Clifton, whence a 40-inch main brought water to North Avenue.

The Gunpowder water was turned in direct to the city supply on September 28, 1881. In 1888 an additional storage lake, holding 265,000,000 gallons of water, was built at Clifton.

It would appear that after 1881, under ordinary conditions, at least seven-eighths of the city's water supply was derived from the Gunpowder River and not more than one-eighth from the old Lake Roland supply obtained from Jones' Falls. A comparatively small area in the southeastern portion of the City was supplied up until 1885 by the Baltimore Water and Electric Company.

In 1876, following a very severe and fatal outbreak of so-called typho-malarial fever, but which was probably paratyphoid, Health Commissioner Steuart employed Professor William P. Tonry to make chemical analyses of the water

[281] of suspected wells in this locality. The wells were found to be grossly polluted. In 1878 and 1879, Dr. Tonry again examined the water of a number of wells in different parts of the City and condemned a large proportion of them on account of pollution with organic matter. In 1885, he examined the waters of numerous wells in the City for the Commissioner of Health and condemned nearly all of them as grossly polluted. He also made chemical examinations of eight samples of mixed water from the Jones' Falls and Gunpowder River water supplies, taken from the city taps, and found them all suspicious. The Commissioners of Health, between this time and 1888, condemned a large number of springs and wells within the City and had them closed, so by the time of the annexation in 1888 there were comparatively few remaining wells in use within the then old city limits.

By the annexation of 1888, the City took over, especially on its northern boundary, a large territory rather thickly inhabited, the water supply of which was almost entirely derived from wells and springs. There was an almost complete absence of sewers of any kind in parts of this territory, especially in the Hampden-Woodberry district. In the first year of annexation, 15 cases of typhoid fever occurred in a locality in Woodberry in which the water supply was obtained from a single well. The city water supply was brought into this new territory, and the wells and springs were condemned and abandoned as rapidly as possible. As late as 1897, Dr. Stokes found on examination a large number of polluted wells in the Hampden-Woodberry district.

In 1892, Commissioner of Health McShane established a sanitary inspection of the Lake Roland watershed in order to reduce pollution of the water supply from this source, and in 1896 this inspection was extended to the Gunpowder watershed, and the pollution of these waters from the privies of private dwellings was, to a considerable degree at least, abated. In his report of this year, Commissioner McShane expressed the opinion that the general water supply was seriously polluted.

In this year the chemical and bacteriological laboratories were established in the Health Department, and from this date

serious studies of the water supply have been made in both [281]
these laboratories.

In general it may be said that chemical and bacteriological examinations made in the Health Department, during this period, of the water of the Gunpowder River and of Jones' Falls and of certain of their larger tributaries, showed an increasingly high degree of pollution, indicated by the presence of organic material and high chlorine content, high colony counts, and the frequent presence of *B. coli;* that there were evidences, both from chemical and bacteriological standpoints, of pollution of the water impounded in the city's reservoirs; and that the water drawn from the city taps showed decided improvement over the reservoir water. In other words, the gross contamination of the water at its source was, to a considerable degree, modified by storage in the city's reservoirs. Dr. Stokes' reports show that there was a rather steady increase in pollution, as judged by the colon bacilli content of samples taken from city taps in 1897 to 1910, inclusive, from 4.5 per cent total samples in 1897 to 57.3 per cent total samples in 1910. He gives the average for the fifteen years, 1896 to 1910, inclusive, as 73.3 per cent of positive colon bacilli findings in 854 samples analyzed. The average bacterial count per cubic centimeter of the tap-water from 1896 to 1902, inclusive, was 600 bacteria. In 1907, it increased to 1300; in 1908, it had dropped back to 600; in 1910, the year of a very considerable typhoid fever epidemic, the average bacterial count per cubic centimeter was 544, and 57.3 per cent of 68 samples examined for colon bacilli were positive. In the previous year, 75 per cent of 112 such samples were positive.
It would appear that a considerable number of wells and [282]
springs were still in use in the City as late as 1910, for in this year Dr. Stokes reports the examination of 71 samples of such waters in which colon bacilli were demonstrated in 64.7 per cent.

In a valuable paper published in 1911 on *The Problem of Typhoid Fever in Baltimore,* Dr. W. W. Ford stated that he had examined samples of the Gunpowder River water, over a period of many years, and especially from October, 1910, to June, 1911, and had found high bacteria counts and demon-

[282] strated the presence of *B. coli* with great regularity. Ford was able to isolate *B. coli* in the majority of 1 c. c. samples, frequently in 0.1 c. c. and more rarely in 0.01 c. c. samples. He found that other intestinal organisms were also commonly present. The contamination of the water was especially marked in the dry weather of 1910.

As will be seen later, Dr. Jones had reached the conclusion in 1907 that the water must be responsible for at least the majority of typhoid fever cases in Baltimore. Dr. Stokes and Dr. Ford, in their studies of the situation reached the same conclusion in 1910 and 1911.

Purification of the city water supply, through the action of calcium hypochlorite, was begun in June, 1911. The hypochlorite was added to the water of both the Lake Roland and the Gunpowder River supplies at the gate chambers through

TABLE 1

THE LAKE ROLAND SYSTEM

		Average bacteria per c. c.	Percentage of bacterial reduction	Percentage of positive colon tests		
				0.1 c. c.	1 c. c.	10 c. c.
July	Raw water	47,600	....	60	86	100
	Storage	725	99	26	57	84
	City taps	4,410	91	0	22	64
August	Raw water	38,540	....	76	..	..
	Storage	1,210	97	32	66	..
	City taps	550	99	3	40	..
September	Raw water	15,095	....	46	80	..
	Storage	286	98.5	4	31	..
	City taps	170	99	6	30	..
October	Raw water	17,830	....	91	100	..
	Storage	457	99.6	7	37	..
	City taps	538	99.5	13	33	..
November	Raw water	58,045	....	36	91	..
	Storage	627	98.9	0	19	..
	City taps	1,430	97.6	4	26	..
December	Raw water	6,540	....	20	35	..
	Storage	583	91	0	12	..
	City taps	642	91.1	5	10	..

Available chlorine 0.4 parts per million during June and July; 0.6 parts per million during August; 0.75 during September to October 15; 1.0 from October 15 to December 31.

which the water passed from the impounding reservoirs to the city reservoirs. The addition of chlorine to the general water supply was followed by a marked diminution of the amount of bacterial pollution, as shown both by the bacterial counts and percentage of colon bacilli findings. In this year there was also a decided reduction in the typhoid morbidity and mortality in Baltimore. Stokes, Hachtel, and Freas studied exhaustively the effect of calcium hypochlorite upon the Baltimore water supply in 1911 and published a paper which is an important contribution to our knowledge on the subject (see Tables 1 and 2). [282]

In 1910, the City began to carry out the plans of eminent engineers to increase the Gunpowder water supply by the erection of a higher dam and the creation of a larger impounding reservoir at Lock Raven and to purify this supply by

TABLE 2

THE LOCH RAVEN SYSTEM

		Average bacteria per c. c.	Percentage of bacterial reduction	Percentage of positive colon tests		
				1/10c.c.	1 c. c.	10 c. c.
July	Raw water...	60,800	....	62	88	100
	Storage	1,835	97	23	56	89
	City taps....	1,580	98	12	29	62
August.....	Raw water...	39,560	....	89	..	..
	Storage	2,497	94	14	48	..
	City taps....	134	99.5	11	37	..
September..	Raw water...	12,409	....	74	..	..
	Storage	754	94.1	14	39	..
	City taps....	336	95.9	0	33	..
October	Raw water...	4,260	....	82	..	..
	Storage	638	98.6	13	39	..
	City taps....	596	99	2	33	..
November ..	Raw water...	129,996	....	55	..	..
	Storage	1,227	99.1	7	20	..
	City taps....	1,085	99.2	8	38	..
December ..	Raw water...	19,313	....	29	92	..
	Storage	1,916	90.1	5	22	..
	City taps....	1,227	93.7	0	12	..

Available chlorine 0.4 parts per million during June and July; 0.6 parts per million during August; 0.75 during September to October 15: 1.0 from October 15 to December 31.

[282] appropriate filters established near the Montebello Reservoir. This work was completed and the filtration plant put into operation on September 15, 1915. At this date, too, the Lake Roland water supply was abandoned, though the works are retained for use in case of emergency. For a few weeks in the winters of 1916 and 1917, on account of necessary repairs to a tunnel, this supply, after heavy chlorinization, was used. For at least two years after the new water supply became available, studies in the bacteriological laboratories showed that frequently colon bacilli were present in the water from city taps in 10 c. c. samples, and not infrequently in samples of even 1 or 0.1 c. c. In some months, over 25 per cent of the 10 c. c. samples of tap-water showed the presence of *B. coli.* These findings in the tap-water were often, indeed almost constantly, at variance with the results of the bacteriologist at the water-works. In the last two years, the colon bacillus determinations, obtained in the City Bacteriological Laboratory, have been relatively low. If low colony counts and low percentage of colon bacillus findings mean water free from typhoid bacilli, it is difficult to see that the water can now play any large part as a bearer of typhoid bacilli in the old twenty-four wards of the City. It seems clear that since the
[283] beginning of the use of chlorine purification in 1911, the water supply has become a decreasingly important source of typhoid infection in these wards. Valuable aids to filtration in guarding the city water supply from infection of typhoid bacilli are inspections of the watersheds by the Water Department and the cooperation of the State Board of Health in notifying the Water Department of the presence of reported cases of typhoid fever on the watersheds and in taking appropriate measures to prevent the pollution from them. The map of these two watersheds, separated by a ridge of hills, shows both to be the seat of numerous larger and smaller settlements, many of them villages of considerable size. Inspection shows that many of the smaller, and all of the larger, tributary streams serve as sewers of direct discharge for human excreta from villages, small settlements, and isolated houses and institutions, and that human waste from many dwellings reaches them indirectly from pipes or surface washing. There are a

great number of stables for horses and cattle, as well as pig- [283] styes, on their banks, and many stableyards and paddocks straddle the smaller streams. While a great deal has been done in the last few years by the State Board of Health to enforce measures which have decreased the pollution from the larger settlements, it is evident to any thoughtful observer that, unless some unforseeable revolution occurs in the methods of disposal of human waste, it is hopeless to expect that in any reasonably near time the water from either of these sheds will be safe for drinking purposes without treatment.

PAVING AND STREET CLEANING

As early as 1782, grading and paving of the streets and sidewalks was undertaken in a systematic manner, under a special commission appointed by Act of the General Assembly. The work was energetically carried out. The street pavements, at first confined to the business section, were of rough cobblestones, and this character of pavement was in general use until 1911, except where Belgian blocks and occasionally asphalt were substituted on a few business streets and prominent thoroughfares. The report of the City Engineer in that year showed the following street mileage of each material: cobblestone, 354.82; Belgian block, 43.49; sheet asphalt, 19.23; vitrified brick, 19.15; wooden block, 1.63; ordinary macadam, 56.01; bituminous macadam, 2.73; bitulithic, 10.10; cement surface, 2.01; unpaved, 57.73. Much of the better paving had been done in connection with the rebuilding of the section of the City burned in the great fire of 1904.

Since 1912, under the stimulus of Mayor James H. Preston and following the building of the new sewerage system, many miles of streets have been regraded to facilitate surface drainage and paved with smooth pavements. Following this, the greater part of the private alleys, which since the early history of the City had been unpaved or paved with cobblestones and were so rough and irregular as to render drainage and cleaning almost impossible, was brought to proper grade and smoothly paved with substantial material. But for the war, this work would have been completed in most of the old twenty-four wards in 1918.

[283] In the meantime, the Street Cleaning Department had been increased in personnel, equipment, and efficiency for the cleaning of streets and alleys and the prompt removal of garbage. As a result of all these improvements and the practical elimination of the cow stables within the City and the substitution, to a great extent, of the automobile for the horse, the stable and the house fly have been greatly diminished in numbers.

MILK AND OTHER FOODS

As in other cities, so in Baltimore, in the early stages of growth, the milk supply was obtained from cows kept in stables and on lots within the city limits. With growth, the neighboring and later, especially with the development of railroads, the distant suburbs were called upon. Speaking by and large, as a natural product, the city and nearby milk were probably of better quality than that from a distance. With city growth and crowding, the feeding of garbage and distillery slop to cows, and the many and rapidly increasing chances of contamination with the microorganisms causing diseases in man, and notably those concerned with diseases of the alimentary tract, especially typhoid fever and dysentery, the city produced milk became not only poorer in quality, but more and more dangerous.

The first recorded investigation of Baltimore's milk is that of Professor Tonry, made at the instance of the Commissioner of Health in 1873. He showed that the best milk came from "dry fed" cows in good stables within the City and immediate suburbs; next stood the farm milk brought in by railroads; and lastly, the milk of the badly stabled, sickly, slop fed cows within the City. Evidence of watering of milk was convincing.

In 1879 an ordinance on milk was passed, making it unlawful to mix water, or any drug, or other article, with milk offered for sale, but there was no provision for its enforcement.

In spite of the recommendations of the Health Department, especially in 1887, when attention was called by the Assistant Commissioner, Dr. McShane, to the milk epidemics of typhoid fever, tabulated by Ernest Hart, no steps were taken by the city authorities to give the Health Department power to control the milk supply until 1894. Soon after the passage [283] by the legislature of an act granting the necessary power, the Mayor and City Council passed an ordinance on May 15, 1894, providing for the inspection of milk and other foods and authorizing the appointment of a chemist and three inspectors. According to the milk standards set by this ordinance, there could be sold in Baltimore only pure, unadulterated, unsophisticated milk, the natural product of healthy cows, not deprived of any part of its cream, and to which no additional liquid or solid or preservative had been added, and which at 60° Fahr. shall have a specific gravity of not less than 1029, total solids, 12 per cent, and butter fat, 3 per cent.

In 1896 an ordinance regulated the ventilation, flooring, and cleanliness of cow stables; required the tuberculin test for milch cattle within the City; and made compulsory the prompt reporting to the Health Department not only of the occurrence of "contagious and infectious" diseases among cows, but of cases of asiatic cholera, croup and diphtheria, [284] measles, scarlatina, smallpox, typhus and typhoid fevers upon premises with milch cows, or where milk is handled or offered for sale. Milk from premises with cases of any of these or other infectious diseases could not be sold or given away until the Commissioner of Health deemed it safe. Persons connected with dairying and milk handling were forbidden to enter such premises.

By the ordinance of 1902, the sanitary standards under which milch cattle could be kept were made more stringent by requiring non-absorbent floors in stables, with proper drainage and more air space and range for cows, and by forbidding privies, cesspools, and the presence of fowl, swine and horses in stables or connected with rooms where milk and cream were handled.

In 1908, a very sweeping milk ordinance was passed, giving the Health Department complete control over the milk supply, requiring permits for handling milk, dairy farm inspection, the sterilization of utensils, regulation of conditions under which milk could be sold, excluding for sale the milk of slop fed cows, and raising the standard for total solids to 12½ per cent and of butter fat to 3½ per cent. In all respects

[284] except one it was a model ordinance, and during the next few years a far-reaching scheme for the sanitary control of milk was put into operation. The weak point of the ordinance lay in its general terms. The regulations made by the Department, especially in regard to sanitary conditions under which milk was handled, sold, and pasteurized, were often successfully contested by certain types of milk dealers as unreasonable, and the Department, which was well and intelligently organized for the purpose, was continuously held up in important details by suits brought to restrain its actions. Conditions, especially in regard to the relation of milk to the spread of communicable disease, were undoubtedly greatly improved. Those concerned in the administration of this ordinance deserve high praise.

Between 1908 and 1916, great changes took place in the milk business, especially in regard to the proportion of milk pasteurized and the attitude of the dealers to the milk problem. In 1917, there was passed an amending ordinance regulating specifically and in detail the important points of administration found impossible of execution under the more general ordinance of 1908. The ordinance of 1917 was passed as drawn by a committee headed by Dr. William H. Welch, and though put into execution under conditions of great disadvantage, it has finally met the best hopes of its authors. It is within the bounds of accuracy to state that all the milk used in the City is reasonably safe from typhoid infection. The great bulk of the milk is pasteurized with fair efficiency, put into bottles or other containers well washed, and, on the whole, if not absolutely sterilized, fairly safe from typhoid infection. The small quantity of raw milk of special grades allowed to be sold has stood the test of experiment without transmitting typhoid fever, and there is every reason for concluding that it is effectually safeguarded. In 1918 and in 1919, no cases of typhoid fever were traced to milk. Certainly there were no ascertainable milk outbreaks such as so often occurred in previous years.

The new milk ordinance did not go into effect until November, 1917, and, on account of war conditions, it was not possible for many dealers to secure and install pasteurizing and other apparatus until well into 1918. Labor for working the plants and ice for refrigeration were also difficult to obtain. It was not until 1919, therefore, that the real advantage of the administration of the ordinance, as shown in the reduction in bacterial counts of the milk supply, was gained. The results for pasteurized milk as delivered from street wagons are illustrated in Table 3 and in the following figures, expressed in terms of annual Levy rating of bacteria per cubic centimeter for the total milk supply: 1917, 500,000 (1959 samples); 1918, 600,000 (2741 samples); 1919, 67,000 (3899 samples).

TABLE 3

LEVY AVERAGE OF NUMBERS OF BACTERIA PER C. C. IN SAMPLES OF PASTEURIZED MILK (ABOUT 300 MONTHLY) COLLECTED FROM WAGONS ON THE STREET

Month	Levy average of numbers of bacteria per c. c.		
	1918	1919	1920
January	282,000	56,000	13,000
February	475,000	41,000	8,300
March	450,000	54,000	15,000
April	650,000	52,000	......
May	1,500,000	96,000	......
June	1,200,000	130,000	......
July	1,500,000	120,000	......
August	1,700,000	110,000	......
September	840,000	120,000	......
October	350,000	74,000	......
November	115,000	14,000	......
December	71,000	27,000	......

Judging from the grossly insanitary conditions under which milk was produced and sold in Baltimore from the earliest times and from the number of milk outbreaks recorded in the reports of the Health Department, it is certain that this article of food has until recently played a very important rôle in the spread of typhoid fever in Baltimore. It is likely that the interference with the watering of milk which attended the enforcement of the ordinance of 1894 was, to a considerable degree at least, responsible for the fall in the typhoid rate during the next few years. It was not, however, until

[284] the enforcement of the new ordinance of 1917 that milk, as a vehicle of infection for typhoid fever, was practically eliminated.

Of the other foods than milk, butter, cheese, shell-fish, and vegetables and fruits eaten raw are the only ones of importance, from the practical standpoint, in regard to possibility of carrying typhoid fever infection. Control of these by the Health Department has been limited to preventing those sick with typhoid fever, those attendant upon them, and known carriers from handling food or selling or giving it to others.

No outbreaks of typhoid fever spread by any of these substances have ever been convincingly traced by the officials of
[285] the Health Department, but it is quite probable that by some of these means, notably butter and shell-fish, especially oysters, typhoid infection has been carried here. Instances of the spread of typhoid fever in a large city, in either isolated cases or in epidemic outbreaks, by means of butter, cheese, and vegetables, would in the nature of the case be hit upon only by chance. Vegetables grown on ground fertilized by night-soil were sold in Baltimore for many years and perhaps are now, but there are no observations upon the relation of this circumstance to the spread of typhoid fever in the community. The finding of *B. coli* on such vegetables means nothing in this relation, for it would be strange if this organism were not present on the surface of all vegetables exposed to the soil and not protected by pods like peas and beans. Oysters from polluted waters have undoubtedly been sold in Baltimore and typhoid fever may well have been spread by this means in years past; but the height of typhoid prevalence has not synchronized with the oyster season since 1881.

STUDY OF TYPHOID FEVER IN THE HEALTH DEPARTMENT

It now becomes our task to consider, as fully as may be, the studies on typhoid fever made in the Health Department.

The first mention of the etiology of typhoid fever in the Health Department reports occurs in 1875 when Commissioner Stewart attributed this disease and certain others to sewer gas entering houses from imperfectly trapped sewer wells, just as Thomas H. Buckler, in 1851, had blamed the cholera and typhoid fever at the Almshouse on the foul gas of an [285] open cesspool. In 1876, Commissioner Stewart attributed an outbreak of "typho-malarial fever" at Fell's Point to polluted wells and had them closed. Three years later, he ascribed the typhoid fever occurring along Harford Run to the foul condition of that sewer stream. Again, in 1880, he blamed the disease on sewer gas, after his inspectors, who had been sent to visit all houses harboring fatal cases of typhoid fever, reported that they had invariably found full or overflowing privy wells or faulty water closets. As a result of this conclusion, very generally accepted at the time, Dr. Stewart got plumbing inspection established in 1885. In the meantime he had bent every effort to control the nuisance involved in the removal of night-soil. At this time, too, he started routine chemical examinations of the waters of springs and wells, a number of which he had condemned. He also began to question the safety of the general water supply. In 1888, Dr. Stewart closed many wells in the Hampden-Woodberry district of the newly annexed territory on account of pollution, having traced 15 cases of typhoid fever in this district to one well.

Dr. George H. Rohe, in his report for 1890, gave more information concerning typhoid fever in Baltimore than all previous Health Commissioners. After noting the progressively increasing number of deaths during the previous nine years, he pointed out that the great excess of cases in the twenty-first and twenty-second wards was due to the polluted wells in the newly annexed territory and further stated that the sanitary inspectors reported that many residents contracted the disease out of the City, particularly at health resorts, and that many non-residents were brought into the City to the hospitals for treatment. The truth of the last statement can be vouched for by all who were associated with Baltimore hospitals at that time.

In 1894, typhoid fever together with certain other diseases was made reportable to the Health Department.

Commissioner McShane, in his report for 1895, devoted considerable space to typhoid fever. He recognized that the disease is not contagious in the usual strict sense of the term, but certainly commonly communicated through infected foods

[285] and drinks. He recommended that every precaution should be taken to guard the water supply. In this year, the City Chemist attributed 12 cases of typhoid fever in an institution to infected milk, and the epidemic subsided after the milk supply was changed. Dr. McShane also took steps to stop the handling of milk by persons associated with cases of typhoid fever and the sale of milk from premises harboring cases of this disease. He stated in 1897 that, " 60 per cent of the cases of typhoid fever, concerning which an intelligent history could be gotten, were infected outside of the City."

In 1899, Commissioner Jones made the first spot map, giving the location of reported cases of typhoid fever and showing that the disease was rather evenly distributed over the City. This point was illustrated in his maps for 1902 and succeeding years. In 1899 and in other years, particularly in his elaborate study of 1907, Dr. Jones reached the conclusion that though flies and milk did play a part in spreading typhoid fever, they could not explain the bulk of the cases for obvious reasons, for too many cases occurred in the months without flies and the cases, in the average years, were too well scattered to be explained satisfactorily by milk infection. He was alive to the importance of this latter means of transmission, however, and recorded three striking milk epidemics: one in 1903 among the workers of a large factory who drank milk supplied to them at luncheon (there were 26 cases with 4 deaths); a second in 1906, an extensive epidemic in the Hampden-Woodberry district; and a third in 1907, a small epidemic of 19 cases.

Contact infection as evidenced by secondary cases in households fell far short, of course, of explaining the great annual visitation. From Dr. Jones' figures, I estimate that in the years, 1904, 1905, 1906, and 1907, somewhere between 7 and 9 per cent of the cases could be classed under this category. Of 2225 cases investigated from the standpoint of out-of-town infection, it was found that 489, or only approximately 22 per cent of the cases had been out of the City. Therefore, nearly four-fifths of the cases could not have received their infections outside of the City. On the other hand, there were still many polluted wells within the City, especially in

[285] certain parts, and chemical and bacteriological examinations of the general water supply year by year showed increasing evidences of pollution, in spite of sanitary inspections and nuisance abatements along the watersheds. Dr. Jones concluded that the water was the means through which the bulk of the City's typhoid was spread.

[286] Dr. Jones insisted that there were actually many more cases of typhoid fever in the City than were reported. He estimated that in 1906 and 1907, there must have been between 2000 and 2500, instead of the 1000 to 1400 reported. This was based upon the fact that the number of reported deaths calculated on a 10 per cent fatality basis would give these totals. For instance, in 1907 there were 1420 reported cases and 230 deaths, a case fatality rate of 16.3 per cent; on a 10 per cent fatality basis there should have been 2300 reported cases.

This study of typhoid fever by Dr. Jones in the report of 1907 will always stand out as one of the best pieces of work done in the Baltimore Health Department. The task he set himself was to determine the manner in which the bulk of the typhoid infection took place at that date. Recognizing that there were many sub-sources, important enough in themselves, Dr. Jones after careful and judicious weighing of the available evidence, came to the conclusion by orderly exclusion that typhoid fever in Baltimore was at that time chiefly water-borne. Important evidence in support of this conclusion had been accumulated during the preceding years by the chemist, Dr. Lehmann, and the bacteriologist, Dr. Stokes. For the next three years from 1908 to 1910, Dr. Jones exerted himself on the administrative side, as the records show, to eliminate as much as possible the spread of typhoid fever by subsidiary channels; *i. e.,* household contact, flies, and milk.

If there were space, I should like to point out in detail the value of the studies of Dr. Stokes on the water, particularly in connection with the effects of chlorinization and filtration, and the important part he has taken in the whole question of the water supply. Attention should likewise be called to the study of the milk supply and the well directed administration of the details of its control by Drs. Blanck and Hopkins and

[286] their assistants in later years, as well as the pioneer work of Drs. Lehmann and Hoffmann, their predecessors. Dr. Jones left the Baltimore Health Department in the fall of 1915, and took charge of the Bureau of Communicable Diseases of the State Health Department. Here in various ways, particularly in tracing cases reported in Baltimore to sources of infection in the counties of Maryland, and in following up clues, in removing cases from the Baltimore list and crediting them to counties of origin, and vice versa, as determined by the evidence, he continued to play an important rôle in the study of typhoid fever in the City.

Dr. W. W. Ford, in his independent investigations of the milk and water supplies and analysis of the Health Department records of the reported cases and deaths of typhoid fever, confirmed the conclusions of Dr. Jones and Dr. Stokes, and thereby rendered an important public service at a critical time when both the public and the medical profession were apathetic.

In 1909, the mortality rate per 100,000 fell to about 24, the lowest in the recorded history of the City, but in 1910, it jumped to over 42, about the level of 1907. As I have already stated, the treatment of the water with hypochlorite of lime and later with alum in addition was begun in June, 1911, and the typhoid rate again dropped abruptly in 1911 and 1912. Since this period there has been a continuous fall in the rate to the present level (in the old city wards).

But it must be pointed out at the same time, that during this period from 1911 to 1919, other factors besides purification of water by chlorinization and filtration have come strongly into play. First in importance perhaps is the doing away with great numbers of privies, a considerable administrative feat on the part of the Health Department, by forcing connections with the new sanitary sewerage system; second, efforts on the part of the Health Department to restrain fly-borne typhoid; third, progress in curbing infection through milk; fourth, education of the public, and to some degree at least, anti-typhoid vaccination; and last, the decline of typhoid in the rural districts, due very largely to the increasing activities of the State Health Department.

THE WRITER'S STUDIES ON TYPHOID FEVER IN THE HEALTH DEPARTMENT

I entered the Health Department in the fall of 1915 and inherited the typhoid fever problem among others from Dr. Jones. The mortality rate per 100,000 for that year was 21.90. During 1916, I could not devote the necessary time to detailed study of histories of individual cases, but I was able to prepare the way for more complete histories than had before been obtainable and to take up more actively than had hitherto been practicable the study of the distribution of cases by five-day periods through a series of fifteen years, as well as to push the investigation of the relation of carriers to milk and to household and institutional epidemics. The latter work confirmed what was already known in the Health Department, that the bulk of the cases and deaths were reported in the summer and fall months, the peak coming usually late in August or in September, the fall in the autumn being gradual, and the rise and fall of the peak extending, as a rule, from July 15 to November 15. There was in the fifteen years rarely any evidence of a marked rise in spring, associated with freshets and so characteristic of the Great Lakes cities. With the greater facilities and leisure for this purpose than anyone had hitherto enjoyed, I set seriously to work in 1917 to determine, if possible, the most important sources of typhoid infection under the present conditions. The task was the easier because the number of reported cases to be investigated, as well as the number of deaths, had greatly diminished. The city officials, as well as the citizens, were greatly disappointed that typhoid fever had not almost completely disappeared after the new sewerage system and new water purification system were brought into use. It will be recalled that in the fall of 1915 the new water supply had become available and most of the connectible houses had been connected with the new sewerage system.

When my studies of typhoid fever in Baltimore began *in 1916,* it was estimated that about 60 per cent of the milk sold in Baltimore was pasteurized more or less efficiently. Some of these milk plants were well operated, both as to pasteurization of the milk and cleansing and sterilization of the milk

[319] utensils, including bottles and cans. In many of the pasteurizing dairies, including some of the largest, there were glaring faults. Most of the plants used the *holding system,* and on the whole their results were better than in those depending upon the *flash system.* In some of these latter plants, notably in one in which the proprietor did the pasteurizing himself, the results were very good. Many of the plants were not only hopelessly dirty and unscreened, but were structurally unfit for their purpose. The supervisor of pasteurizing dairies and his assistant, who overlooked these plants for the Health Department, were not only well trained but diligent and tactful. Their supervision consisted of general inspection, the inspection of automatically registered temperature charts, the insistence on proper cooling of milk, and the cleanliness of plants and utensils. They submitted a large number of samples of raw and pasteurized milk to bacteriological examination and spent much time in instruction. They won the cooperation of many of the dairymen, and beyond question, they did much to lift milk pasteurization from a purely commercial basis to a method of disease prevention. A large part of both the pasteurized and raw milk sold was delivered in churns, from which the milk was dipped with hand dippers for domestic use. Much of the milk sold in small grocery and milk stores was similarly served to customers. The condition of the churns and of the refrigerators in many of these stores beggars description. The regulation of the Health Department forbidding milkmen from removing bottles from houses harboring cases of communicable diseases until the bottles have been disinfected by the Health Department was being obeyed. The inspection of milk on its arrival in town to guard against skimming and watering was fairly well covered, as had been the case since the procedure was instituted in 1894. The far reaching system of dairy farm inspection with its cooperative relation with the deputy state medical officers, inaugurated by Drs. Jones and Blanck, was being carried out as well as could be expected.

The anti-typhoid work of the Health Department was extended in 1916 by a search for carriers in households, in restaurants and oyster-shucking establishments, and in con-

nection with dairies and dairy farms. A canvas was made of [319]
restaurants, dairies, and dairy farms for workers who had
typhoid fever. From the large number of such persons thus
apprehended, routine cultures of the bowel and bladder dis-
charges were made. Two carriers were found among dairy [320]
employees within the City. To one of them 10 cases of typhoid
fever were traced. The other was a driver in a milk wagon
who only handled the milk after it was bottled and to whom
no cases of typhoid fever had been traced. Both were with-
drawn from milk work. Workers on dairy farms suspected of
being carriers, either came to the laboratory to furnish the
desired specimens for culture, or the specimens were collected
by the deputy state health officers. Two suspected carriers
who had had typhoid the year before refused to be cultured.
An epidemic of 24 cases of typhoid fever among the users of
the milk from the dairy farm on which they lived subsided
at once after the milk from this farm was excluded from the
City. A total of 34 cases of typhoid fever was traced to milk
during 1916. Four cases in one household and 1 case in
another were traced to one carrier, a butler who had had typhoid
fever fifteen years before. Nine cases in a college were traced
to a female cook in a restaurant who proved to be a carrier.
Another carrier, a cook in a private family, was apprehended.
To her were attributed 2 cases of the disease, one occurring
in 1914 and the other in 1915. In all, eight carriers were
apprehended in 1916. Including the milk epidemic presum-
ably due to one or two carriers on a dairy farm, nearly 30 cases
of typhoid were traced to carriers this year.

All the remaining wells within the City were investigated by chemical and bacteriological methods, and quite a number showed evidences of pollution and were closed. Dr. Stokes isolated *B. typhosus* from the water of one such well that had been suspected as the source of infection of 4 cases of typhoid fever. A number of wells at roadside and fishing resorts near the City, frequented by Baltimoreans, were found to be polluted and reported to the State Board of Health.

One hundred and twenty-five cases of typhoid fever were classified as "infected out of town." Some of these were residents of the City who had contracted the disease while

[320] away for work or pleasure—many of them at summer resorts. Nine out of fifteen members of a baseball team developed typhoid fever after drinking water from a known polluted source at Grafton, West Virginia. Of the 110 Baltimoreans infected outside the City, 101 were infected in rural Maryland, and of the whole number of such outside infections, 106 occurred in the period of greatest incidence of the disease, as follows: July, 19, August, 23, September, 16, and October, 18.

Of the 776 reported cases, a satisfactory explanation of the source of infection was traced in 178, or nearly 22 per cent.

In addition to a search for carriers, attempts were made for better control of the milk supply, the elimination of polluted wells, and the prosecution of the fly nuisance through the newspapers, various civic bodies, certain insurance companies, the Street Cleaning Department, the health wardens, the nurses, and the Police Department. Instructions as to how to avoid typhoid fever, both at home and abroad, and urging anti-typhoid inoculations were not only distributed in the homes of cases of the disease, but through the medium of the newspapers, and circulars left in the houses. The latter was accomplished very largely through the collecting agents of industrial insurance companies. Physicians were requested to urge the general use of anti-typhoid vaccine, especially in households with recent cases of disease. The time was ripe this year, too, to prosecute the grading and smooth paving of the private alleys of the City, so long a nuisance in promoting the breeding and feeding of flies. This work, begun in 1916, was more than half completed with the paving of about one half of the 3000 such alleys, when the work was temporarily abandoned on account of the war.

In *1917*, the mode of infection was more or less convincingly traced in 225, or 41.17 per cent of the total number of 544 reported cases. The seasonal distribution of both cases and deaths remained relatively the same as in previous years. One hundred and forty-two, or 26.10 per cent of the reported cases, were classified as infected outside of the City (14 of these came from ships); 75, or 13.78 per cent of the total number of cases, were infected in rural Maryland; and 53, or

9.74 per cent, contracted the disease in other states. Table 4 [320] gives the distribution of these cases according to counties and according to other states. A considerable number of the 13 cases attributed to Pennsylvania occurred in non-residents of Baltimore, who came here for medical treatment or for work and who were either sick at the time of their arrival or became so within a day or two thereafter. Of the 13 cases attributed to Virginia, 4 were among Baltimoreans who became sick at Virginia Beach, and several other patients came to Baltimore from Norfolk or Portsmouth to hospitals for medical treatment. Of the 75 cases attributed to rural Maryland, a very large proportion (44) contracted the disease while sojourning in Baltimore and Anne Arundel Counties for work or on vacation. Nearly all of these cases occurred in the warm weather, when typhoid fever is most prevalent in the country districts. Of the total number of cases, 43, or 7.9 per cent, were traced to milk, there being three clear cut milk epidemics during the summer, all of which were traced to infection of the milk in dairies within the City. In 15 cases, a history of contact with recent cases within the household was obtained, and in 6 further cases the infection was traced to hospitals [321] in Baltimore, making a total of 21, or 3.85 per cent of cases

TABLE 4

IMPORTED CASES AND OUT-OF-TOWN INFECTIONS, 1917

Maryland counties		Other states	
Baltimore	29	Massachusetts	1
Anne Arundel	15	Maine	2
Kent	9	West Virginia	2
Harford	4	Delaware	3
Worcester (Ocean City)	4	North Carolina	3
Caroline	3	New York	7
Frederick	3	New Jersey	9
Dorchester	2	Pennsylvania	13
Carroll	2	Virginia	13
Charles	1		
Prince George's	1		53
Wicomico	1	Ships	14
Allegany (Cumberland)	1		
			67
	75		75
			142

[321] traced to contact with clinical cases of typhoid fever. Fifteen cases occurred in families in which one or more members, occasionally unrelated inmates, had had typhoid fever one or more years before—the interval ranging from thirty years to one year. These previous cases of typhoid fever occurred in mothers, five times; grandmothers, twice; sisters, twice; brothers, four times; and fathers and wives, once each. In several of these families, there had been repeated cases. In two of the fifteen families, carriers were demonstrated by culture methods. One of these families is of particular interest, there having been, during a period of seventeen years, four outbreaks of typhoid fever in three generations with a total of 8 cases. The grandmother had contracted the disease seventeen years before; the next year, her two sons, her son-in-law and daughter and an unmarried daughter, all living in the same house with her, developed typhoid fever. In June, 1916, this woman's grandchild, aged 7 years, who lived in her house, developed the disease. On January 2, 1917, a second grandchild, a sister of the first, was reported as a case of typhoid fever. The whole family was cultured on January 8, 1917, and the grandmother, the unmarried aunt, and the child who had had typhoid fever in June of the previous year, were all three found to be carriers. Another carrier was found in the person of the mother of a boy who had had typhoid fever the previous year. In this year, eleven additional proven carriers were added to the Health Department's list, making a total of nineteen carriers under observation. In connection with contact infection, 4 cases gave a history of having had typhoid fever before, and it is suggested that these were examples of self-infection. One of these cases occurred within eight months, and a second within twelve months of the primary attack. One of the cases was treated in both attacks at the Johns Hopkins Hospital, the first attack occurring in August, 1908, and the second in January, 1917. In the fourth case, the attacks were six years apart.

In 1917, the morbidity rate per 100,000 was 83.79. Notwithstanding the fact that 24 deaths, or 26 per cent of the total of 92 deaths, occurred among typhoid fever patients brought to Baltimore hospitals for treatment from rural Maryland, the mortality rate fell to 14.17 per 100,000. There [321] were 152 cases officially recorded by the State Board of Health against the counties from which they came, among whom the cases fatality rate was 15.85 per cent. The annual case fatality rate, excluding the above, but including cases and deaths of persons from other states than Maryland, was 11.75 per cent.

In *1918,* there were 302 cases and 73 deaths from typhoid fever, a decrease of 45 per cent in the cases and 28.7 per cent in the deaths as compared with 1917. The distribution of cases and deaths by months followed the same course as in previous years, with the exception that during October, the period of the influenza epidemic, the number of deaths from typhoid fever was disproportionately high, there being a total of 17 deaths for this month against 11 in September and 1 each in November and December. The number of out of town infections of Baltimore residents on trips, and of imported cases, was 53, or 17.55 per cent of the whole. In 39 cases, or 12.58 per cent, infection was attributed to contact with recent cases in the households. Five cases in one family were traced to a carrier and there was one case of laboratory infection. In addition to these, 16 cases were probably infected outside the City, and in 20 cases this mode of infection was a possibility. Twenty-two cases, or 7.9 per cent of the total number, resided in the same house with persons who had had typhoid fever previously, at times varying from thirty years to one year. Of the 24 possible infectors for this group of cases, there were 16 females and 8 males, but 2 of the possible male infectors were associated with possible female infectors in the same house; that is, in two of the households, the father and sister of the patients had had the fever. The 16 possible female infectors showed the following relationships to the patients: mother in 9 cases, sister in 3, wife in 1, unrelated female in 3 cases. The relation of the male infectors to the patients was: father alone, twice; father with sister, twice; brother, twice; husband, once; unrelated male, once. One father, upon whom the carrier state was proven by culture, was probably responsible for 5 cases among his children. In the latter part of July and the early part of August, the five children of a

[321] police officer came down in rapid succession with typhoid fever. The father, who had had typhoid fever fifteen years before, proved on culture to be a fecal carrier. No other source of infection for these children was ascertainable. A large proportion of these probable carriers were cultured, but only the one above mentioned was proven to be a carrier. Two additional carriers, to whom no cases were attributed, were apprehended. This year again, 4 cases presented a previous history of having had typhoid fever. The case of laboratory infection occurred in a medical student who swallowed typhoid bacilli sucked up through a pipette from a bouillon culture while working in a bacteriological laboratory.

The morbidity rate fell to 45.82 and the mortality rate to 11.08 per 100,000. Fourteen, or 19.18 per cent, of the 73 deaths from typhoid fever registered against Baltimore occurred among patients from rural Maryland sent to the Baltimore hospitals for treatment. There were 94 such cases against the 152 in the previous year. The case fatality rate among these cases was 14.9 per cent. During 1918, there were 59 deaths from typhoid fever properly chargeable to Baltimore, giving a case fatality rate of 17.2 per cent. As previously pointed out, the case fatality rate was influenced in great degree by the influenza epidemic in October, when there were 17 deaths among typhoid fever patients.

In *1919,* there was a large annexation of territory, in part closely built up, with a population for which no accurate figures are as yet obtainable. On the basis of the police census taken in the spring of 1919, it was estimated at 73,000, the proportion of negroes being much smaller than in the old City. The Bureau of the Census authorized the Health Department, for statistical purposes, to add about 60,000 for the annexed territory to the estimated population of the old
[322] City. As the inclusion of this territory and its unknown population in the last year of the series would make accurate comparison of the course of the disease in former years impossible, for the purposes of the present study, the figures for the old City (old twenty-four wards) and for the Annex (new four wards) have been kept separate. The figures used in the graph are those derived from the old twenty-four wards.

The problems of typhoid fever in the annexed territory will, [322] therefore, be considered separately.

In the old twenty-four wards, the distribution of cases and deaths by months did not vary materially from the usual. Of the 293 reported cases, the modes of infection were traced as follows: 50, or 17.06 per cent, including definite imported cases in non-residents of Baltimore, and Maryland outside of Baltimore, were infected outside of the City; 36, or 12.78 per cent, occurred as secondary infections from recent previous cases in households and hospitals; 12, or 4.09 per cent, were infected by polluted wells and streams (2 cases were traced to wells at Curtis Bay and 10 to drinking the water of Gwynn's Falls); and 2 cases, or 0.67 per cent, were traced to laboratory infection. A total of 100 cases, or 34.05 per cent of the whole, were explained with reasonable certainty. A probable explanation was obtained for 52, or 17.73 per cent of the cases: 23, or 7.84 per cent, due to infection from remote cases in households; 5, or 1.70 per cent, due to neighborhood infection (from recent cases, probably through the medium of flies); and 23, or 8.19 per cent, due to infection outside the City. In addition, there were 14 cases, or 4.74 per cent, that were possibly infected outside the City. Altogether, an explanation, more or less satisfactory, of the mode or source of infection was obtained in 166 cases, or 56.52 per cent.

The cases attributed to infection from Gwynn's Falls occurred in ten children, members of a Sunday School in Southwest Baltimore (twenty-fourth ward) who took part in a picnic at Gwynn's Falls Park. Some twenty of the children went wading in Gwynn's Falls and, the day being very warm, drank the water of this stream. The cases of typhoid fever were restricted to those who so used the stream, and careful inquiry revealed no other source of infection. Half of those exposed to this source developed the disease. The stream at this point receives a considerable body of household sewerage, and there had been cases of typhoid fever in this area during the summer. The stream is also open to serious pollution above this point. It is of interest, that, as in 1918, no cases of typhoid were traced to milk. Two carriers were appre-

[322] hended, making a total of twenty-four under restriction by the Health Department.

The morbidity rate was 43.79 per 100,000. There were 49 typhoid deaths in the old city, including 8 non-residents infected outside the City of whom 5 were from rural Maryland. The mortality rate was, therefore, 7.21 per 100,000. The mortality rate, less the 5 deaths among the 34 cases brought from rural Maryland to Baltimore hospitals for treatment, but including 3 other deaths of non-residents (a seaman, a person from Delaware, and another from West Virginia), was 6.58. The mortality rate among Baltimoreans alone was 6.28. The case fatality rate, based upon the 49 deaths occurring in the old twenty-four wards, and especially chargeable against Baltimore, was 16.72 per cent. The case fatality rate among the 34 cases from rural Maryland brought to Baltimore for medical treatment was 14.80 per cent. The case fatality rate for Baltimore minus these 5 deaths was 15.01 per cent. A more accurate picture of the case fatality rate, however, is obtained by dividing the whole number of cases reported in Baltimore in 1919 into the total number of deaths occurring among these cases not within the calendar months, but within a reasonable time afterwards. Of the 293 cases reported 41 died within the year 1919 and 3 between January 1 and May 1, 1920—a total of 44—giving a case fatality rate of 15.01 which happens to be exactly the same as that obtained when the deaths of patients from rural Maryland were left out of consideration. Approximately the same case fatality rate (14.80 per cent) is obtained by dividing the total deaths, including those of all non-residents dying in Baltimore, by the total number of cases reported in Baltimore, plus the cases brought into the City from rural Maryland for treatment (293 + 34 = 327). It would appear that a case fatality rate of about 15 per cent is correct for Baltimore (old twenty-four wards) in 1919.

Just as in the annexation of 1888, so in that of 1919 the City annexed a territory, much of which was characterized by insanitary conditions and with a high typhoid rate. In certain parts of this area, as in Roland Park, sanitary conditions were better than in the City itself. According to data kindly furnished me by Dr. C. Hampson Jones, when Chief of the [322] Bureau of Communicable Diseases of the State Department of Health, there were 128 reported cases of typhoid fever in 1918 in the area annexed in 1919. A large proportion of these cases, 48, were in Highlandtown; 31 were in Brooklyn; and 22 in Curtis Bay. There were 10 cases in Arlington, 6 at Mt. Winans, and 5 at Hullsville. The water supply of Highlandtown is derived from the grossly polluted Herring Run, but is treated with chlorine. Upon examinations made in the laboratories of the Baltimore Health Department during 1919, this water supply showed evidence of marked pollution. In at least one milk epidemic in Baltimore in 1917, studied by me, cases of typhoid fever occurred in Highlandtown among customers of the infected dairy in the old City. There is every reason to suppose that milk has in the past played at least as important a part in spreading typhoid fever in Highlandtown as in the old City. The sewerage system is as crude as it formerly was in Baltimore. Much of Brooklyn and Curtis Bay proper have pure water supplies, filtered water from the Patapsco in the former and artesian well water in the latter. Both are, however, in large part without sanitary sewerage disposal; privies and cesspools being still largely in use. Overflowing privies and cesspools are common. In the summer of 1918, there was an extensive milk outbreak of typhoid fever in Brooklyn and Curtis Bay, traced to a single dairy. Mt. Winans and Hullsville are comparatively small places, in which the water is derived from ordinary wells, and the common privy is still in use. [323] Many of the smaller villages and settlements in the northwestern portion of the annex have pure water, supplied either from artesian wells or from the Avalona supply of the Baltimore County Water Company. Good water is served to Mt. Washington, Roland Park, a part of Arlington and Govans, and to a part of the developments on the Harford Road. In much of Arlington, Govans, and the settlements along the Harford Road, there are no proper sewers, and cesspools and privies are situated so as to pollute the wells from which drinking water is obtained. So, in much of the newly an-

[323] nexed territory, the City is confronted with insanitary conditions fraught with danger.

With the annexation, the City's laws and regulations in regard to milk became immediately applicable to the new wards, and fortunately in 1919 there were no milk epidemics. There was a total of 65 cases of typhoid fever reported in the four new wards in 1919, in comparison with 128 the previous year. Of the 65 cases in the Annex, 8, or 12.31 per cent were classified as infected outside of the City; 6, or 9.23 per cent, were attributed to contact with recent cases in households; the same number, to contact with remote cases; 12, or 18.46 per cent, to infection from wells or springs; and 2, or 3.07 per cent, to neighborhood infection, probably by flies. No cases were traced to milk. It will be noted that in the Annex a somewhat larger proportion of the cases were explained than in the old twenty-four wards.

On an estimated population of 65,000, there was a morbidity rate of 100 per 100,000. There were 9 deaths. The mortality rate was 13.84 per 100,000, and the case fatality rate was 13.84 per cent. The mortality rate of the new four wards was nearly twice that of the old twenty-four wards.

SUMMARY OF INFORMATION IN REGARD TO SOURCES OF INFECTION OF TYPHOID FEVER, 1916-1919

Sources of Infection in Reported Cases.—In Table 5, the results of the investigations of 1916 to 1919, inclusive, on the sources of infection of reported cases are set forth. It will be noted that the proportion of cases for which no explanation of the mode of infection is determined falls steadily from 1916 to 1919. This is to some degree due to the fact that in the first two years the data in regard to probable and possible outside infections, having been destroyed by fire, are lacking in the table.

Attention is directed to the very large proportion of cases certainly infected outside the City, varying from 16 to 26 per cent in the four years. These include the imported cases occurring among non-residents from other states or from foreign countries. The great bulk of them, however, are represented by residents of Baltimore who contracted the disease outside of the City while away for work or vacation. It will [323] be seen that the percentage of cases infected outside of the City is fairly constant for this period. In addition to this group, there are two other comparatively large groups in 1918 and 1919, one in which infection outside of the City was probable but not certain, and a second in which it was possible, due to the fact that the individuals had been out of town for one or two days only within three weeks or a month before the beginning of the illness.

TABLE 5

THE SOURCES OF INFECTION OF TYPHOID FEVER FOR 1916 TO 1919, INCLUSIVE

Sources of infection	1916		1917		1918		1919			
	Cases	Percentage of cases	Cases	Percentage of cases	Cases	Percentage of cases	24 wards cases	Percentage of cases	Annex cases	Percentage of cases
Infected out of city, including imported cases.	125	16.14	142	26.10	53	17.55	50	17.06	8	12.31
Contact with recent cases in households and hospitals.	..	....	21	3.86	39	12.58	36	12.28	6	9.23
Contact with remote cases in households.	..	....	15	2.75	22	7.90	23	7.84	6	9.23
Neighborhood infection.	..	....	.	....	..	....	5	1.70	2	3.07
Springs, wells, and streams.	4	0.51	..	....	..	....	12	4.09	12	18.46
Carriers	14	1.80	..	....	5	1.65	..	....	..	....
Milk	34	4.52	43	7.90	..	....	..	...	..	....
Laboratory	..	....	..	....	1	0.33	2	0.67	..	....
Self-infection	..	....	4	0.73	4	1.32	..	....	..	....
Probable outside	..	....	..	....	16	5.22	24	8.19	8	12.31
Possible outside	..	....	..	....	20	6.26	14	4.74	1	1.53
Unexplained	597	77.13	319	58 83	142	47.02	127	43.34	22	33.84
Total cases	774	100.00	544	100.00	302	100.00	293	100.00	65	100.00

The very much larger percentage of cases traced to contact with recent cases in households and hospitals in 1918 and 1919 over that of 1917 is undoubtedly to some extent due to

[323] more adequate histories obtained in the last two years. Cases were placed in this group only after the most critical consideration; no cases were so assigned unless there was a definite interval of a month or more between the beginning of the illness of the first and succeeding cases, or cases, and only when any other mode of infection could be excluded with reasonable certainty. A surprisingly large percentage of these cases occurred among individuals admitted to the general wards, or semi-private wards, of hospitals to be treated for other affections and who developed the disease three weeks or more after admission. In nearly all of these cases, definite associative contact with typhoid fever cases in the wards could be traced, and in every instance there were cases of typhoid fever in these hospitals at the time of the patient's stay. These cases occurred in four large hospitals.

It will be noted that infection from springs and wells played an unimportant part, there being 4 cases traced to one polluted spring in 1916 and 2 such cases in 1919. In the latter year, there was an epidemic of 10 cases among children who drank the polluted water of Gwynn's Falls.

It is significant that in 1916 and 1917 a large number of cases in definite epidemics were readily traceable to infec-
[324] tion through milk, whereas in the last two years, after the new milk ordinance was put into force, no cases were traced to milk.

The 3 cases traced to infection in bacteriological laboratories in 1918 and 1919 were, of course, fortuitous and have no important bearing upon the question under discussion.

Under self-infection are included the 8 cases noted in the four years, in which the individuals gave a history of having had typhoid fever before and which are included in the table as possibly explained cases. Concerning the possible importance of this source of infection, much can, of course, be said for and against.

There are included in the group under carriers, all cases in which infection through means other than milk is attributed to proven carriers. As will be seen later, although diligent search was made, comparatively few cases in households were traced to proven carriers during the last three years. Allusion has been made to the extensive investigation conducted [324] since 1916 for the apprehension of possible carriers among food handlers. Including these and possible carriers in households, in the four years, 426 individuals were submitted to the test made by culture of both feces and urine. The methods in use for this purpose are those of the U. S. Army Manual; *i. e.,* cultures from fluid stools or from urine on Endo's medium. The work was done by Dr. Stokes or by Dr. Hachtel, or under their immediate supervision. Twenty-four, or 5.26 per cent of these individuals, were thus proven to be carriers. There are now, therefore, twenty-four carriers under control of the Health Department.

Attention was drawn to the importance of infection from remote cases in households when searching for carriers in households, in which, according to the histories of reported cases, a member of the household, almost invariably a relative, was said to have had typhoid fever at some remote date. While from time to time the carrier state was established in many of these suspects by culture, more often even repeated cultures from both bowel and bladder discharges were negative. As my experience with proven carriers, especially bowel carriers, grew, I was struck with the fact that, as tested by cultures made in the ordinary way, there was great intermittency of the carrier state. In the case of a urinary carrier, a butler, there was very considerable intermittence of the emission of the typhoid bacilli. This man served in one family for eight years before any of them developed clinical typhoid fever. Suddenly four were attacked in short order, and his own grandson, who had been living with him for some years, developed the disease at the same time. Further accumulated evidence showed that in a very large proportion of the cases occurring in individuals living in households with proven carriers this association had been going on for years. It seemed to me likely that the infection, or non-infection, of the exposed must be determined by something more than chance contamination of food or drink, under conditions favorable for the multiplication of the typhoid bacilli in such articles to a degree necessary to make an infective dose for the victim, even if it be assumed that the susceptibility of the

[324] latter remained constant. Experience with typhoid carriers shows that in many instances at least a very favorable combination of circumstances, ruled to a very considerable degree by chance, must occur in order that they may successfully inoculate others with the typhoid fever disease. From my experience, it would appear that one of the factors involved in this combination is the intermittence of emission of the infective agent by the carrier. Another forcible factor must be the opportunity offered for infection by the character of the associative contact between carrier and victim. It is well known that carriers who handle food for others have better opportunities than have non-food handlers for transmitting typhoid organisms, at least in numbers adequate for producing clinical typhoid fever. Therefore, suspected carriers, the possible infectors of individuals living in households with them, should be shown to be peculiarly favorably situated in the character of their associative contact to transmit the infective agent, before any definite importance can be attached to them as the sources of infection. Unless this is done, and other possible sources of infection can be excluded with a reasonable degree of certainty, the association between cause and effect is based upon little more than suspicion. If it be shown that the emission of typhoid bacilli (as proved by the culture test) is intermittent among chronic typhoid carriers, and that the suspected carriers, supposed to be responsible for infecting household associates, occupy a relation in the household that favors transmission of the infective agent by food and drink and are in any large proportion of cases food handlers, the character of the evidence is raised to a much higher level.

Analysis of the observed cases during the three years, 1917 to 1919, inclusive, shows that of 68 such cases, including carriers and suspected carriers in households, 66.17 per cent were females, and the percentage of females to males was approximately the same in each year. (See Table 6.)

The forty-five female carriers or suspected carriers bore the following relationships to the typhoid fever patients: Mothers, 24; sisters, 12; wives, 3; grandmothers, 2; aunts, 1; domestic servants, 3. The twenty-three males in question held relationship to the patients as follows: Fathers, 9; brothers, 9; husbands, 3; uncle, 1; unrelated male, 1. [324]

Of the whole number in this group of sixty-eight suspected carriers, in only six cases was the carrier state established by culture.

TABLE 6

SEX DISTRIBUTION OF PROVEN AND SUSPECTED TYPHOID CARRIERS IN HOUSEHOLDS

Year	Number of persons	Females	Males	Percentage of females
1917...........	15	10	5	66.66
1918...........	26	18	8	69.23
1919...........	27	17	10	62.96
	68	45	23	66.17

[325] The argument that these suspected carriers were, at the time of the infection of the individuals associated with them in their households, true carriers and responsible for spreading the disease is supported mainly by the facts; first, that in none of the cases of typhoid fever was any other source of infection traceable; second, that as determined by ordinary culture methods, known typhoid carriers often show marked intermittence of emission of typhoid bacilli in their discharges; and third, that there was a great preponderance among the suspected carriers of mothers and sisters and other females most likely to be food handlers.

If the inference that typhoid bacilli carriers, both fecal and urinary, are often intermittent in the discharge of typhoid bacilli be correct, it naturally follows that it is erroneous and dangerous in public health administration to conclude, on the basis of a single or even several negative cultures, that an individual who has had typhoid fever is not a carrier. It would rather seem that, in order that a typhoid carrier may infect others, among the other various elements of chance that must fall favorably is the one that the carrier be in a period of emission of the infecting agent. It is now well established that, in a typhoid bacillus carrier, cultures may show this organism in large numbers in material removed by a duodenal

[325] tube and yet none appear in fecal cultures taken at the same time. If this be the case with fecal carriers, even much more weight is to be placed upon the evidence at hand in support of the theory of intermittency of the discharge of typhoid bacilli in the case of urinary carriers, for it is a comparatively simple matter to cultivate the typhoid bacillus, when present, from urine collected in a sterile container, for the chances of overgrowth of the typhoid bacillus by other organisms is slight, probably much less than is the case with material collected from the duodenum in bile-system carriers.

TABLE 7

SOURCES OF INFECTION OF TYPHOID CASES FOR 1919

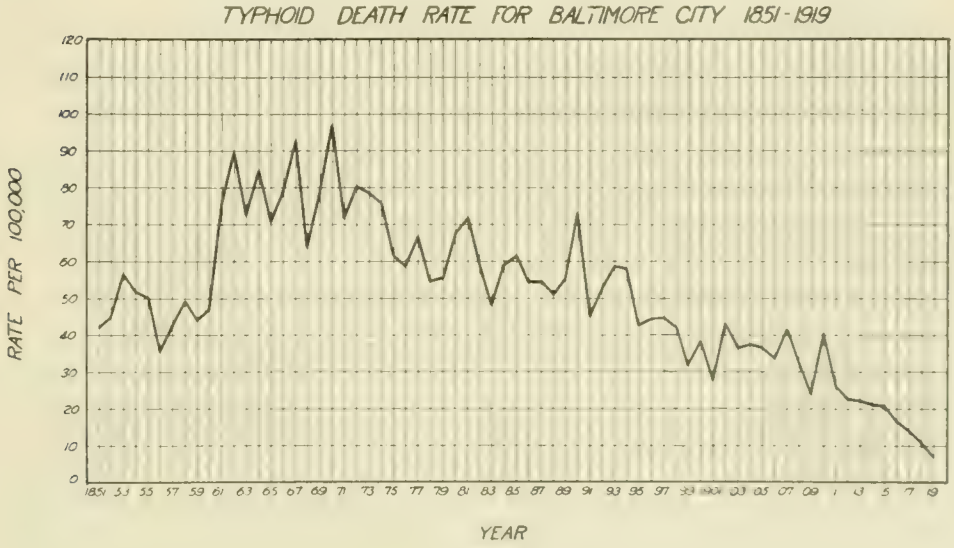
	Old 24 wards		Annex		City as a whole	
	No. of cases	Percentage of cases	No. of cases	Percentage of cases	No. of cases	Percentage of cases
Certainly explained						
Imported	14	4.74	..	....	14	3.91
Outside certain	36	12.28	8	12.31	44	12.29
Recent contact, homes and hospitals.	36	12.28	6	9.22	42	11.73
Laboratory	2	0.67	..	....	2	0.53
Gwynns Falls	10	3.41	..	....	10	2.79
Springs and wells	2	0.67	12	18.46	14	3.91
Total	100	34.05	26	39.99	126	35.16
Probably explained						
Remote cases	23	7.84	6	9.23	29	8.10
Neighborhood infection	5	1.70	2	3.07	7	1.95
Outside probable	24	8.19	8	12.3	32	8.93
Total	52	17.73	16	24.61	68	18.98
Possibly explained						
Outside possible	14	4.74	1	1.53	15	4.18
Total	14	4.74	1	1.53	15	4.18
Total explained	166	56.52	43	66.13	209	58.32
Total unexplained	127	43.34	22	33.84	149	41.62
Final total	293	100.00	65	100.00	358	100.00

In Table 7, the results of the study of the sources of infection of the cases of typhoid fever, reported from the old

twenty-four wards and the Annex during 1919, are set forth [325]
in greater detail.

STATISTICAL ANALYSIS

In this analysis, it is for the most part necessary to proceed under the assumption that the official figures of the Health Department for cases and deaths of typhoid fever are correct. It is realized that this assumption includes many sources of error. Unfortunately, the available data do not afford material upon which even approximate corrections may be based. The chief sources of error affect the period previous to 1890.



GRAPH 1

I. Mortality Rates

Total Population.—In Graph 1 are represented the annual mortality rates per 100,000 living population in Baltimore from 1851 to 1919, inclusive. For the sake of more accurate comparison, the rate for 1919 has been calculated for the old twenty-four wards of the City. It will be noted in the graph that for the first ten years the mortality rate averaged about 45 per annum. In 1861, there was a decided increase in the rate which continued at about 80 until 1871, since which time there has been an irregular but decided fall.

These rates have been calculated from the reported deaths from typhoid fever alone, with the exception of the years from

[325] 1879 to 1898, inclusive, in which the deaths ascribed to "typho-malaria" have been included. If the rates calculated from the deaths from gastric fever be added to the rates given for typhoid fever on the graph, from 1851 to 1875 inclusive, (the years of our period during which gastric fever, which was very likely typhoid fever, played a prominent part as a cause of death), the course of the curve for typhoid fever could not be materially changed; it would simply mean adding rather uniformly from 5 to 10 to the mortality rate from 1851 to 1875. Any important influence of typhus fever on the curve
[326] for typhoid fever is rather remote for two reasons: In the first place, though typhus fever occurred endemically in Baltimore until 1857, its course was marked by distinct epidemic waves, and the wave which began in the early 40's subsided in the early 50's. In the second place, the fact that typhoid fever was first recognized by the Health Department in 1851 suggests very strongly that by this time the diagnosis between this disease and typhus fever was made with a fair degree of facility by local physicians.

The deaths from malarial fevers under various names, including bilious fever, present problems of greater difficulty in this connection. From the early medical literature of Baltimore, it is clear that bilious fever, or bilious remittent fever as it was usually called, was regarded by the medical profession as a form of malaria due to marsh effluvia and as synonymous with remittent malarial fever. It was commonly confused with yellow fever, which was regarded by many of the ablest physicians not as a separate disease but as a severe type of bilious remittent fever. To it was ascribed a very heavy mortality from 1812, when my records begin, until 1842. In 1851, bilious fever declined to 22.53 per 100,000, but during the next three years there was a distinct rise, succeeded by a considerable fall in 1856. By 1861, the rate per 100,000 had fallen to 15. After 1867, there was another rise, culminating in 1874.

If the rates for all the malarial diseases taken together were added to those for typhoid fever, the curve for the latter would show two distinct upward waves; one a very high short one, corresponding to the one evident on the graph between 1851 and 1856; the other a long wave, culminating in a very high [326] peak in 1873. After 1874, the shape of the curve would not vary materially from that of typhoid fever as shown on the graph.

To remittent fever were ascribed comparatively few deaths before 1870, and the highest rate attained by this disease from 1851 to 1870 was 4.88 per 100,000. Intermittent fever was recognized as a separate disease from the beginning of my records for Baltimore. This disease ran a very irregular course, never reaching a very high mortality rate except in 1821 when the rate was 29.85 per 100,000, and, on the whole, its curve rises and falls with that of bilious fever. If all the deaths ascribed annually to intermittent fever were regarded as due to typhoid fever, the rate of the latter, from 1851 to 1866, would not be greatly altered, and its curve would be scarcely affected. Of all the varieties of malarial fever, this one is least likely to be mistaken for typhoid fever. From 1867 to 1874, inclusive, the rate for intermittent fever, and correspondingly the rate for all the fevers ascribed to malaria showed a decided rise.

In the above comparison, it is sought to bring out the facts that, if all the deaths ascribed to the different types of malaria, occurring between 1851 and 1874, inclusive, were classified under typhoid fever, the course of the curve for the latter would be changed in two respects; namely, the upward wave between 1851 and 1856 would be much higher, and the rise in the rates between 1861 and 1870 would have begun several years earlier and continued three years longer—denoting two epidemic waves of typhoid fever instead of one, the peak of the first in 1853 and that of the second in 1871. In reality, however, as the malarial fever deaths between 1866 and 1874 were ascribed chiefly to those types of the disease least likely to be confused with typhoid fever (intermittent and congestive fevers), the confusion of malaria with typhoid fever as a cause of death could not very materially have affected the curve as plotted from the deaths officially ascribed to typhoid fever. Nevertheless, two points are evident: First, that typhoid fever before 1851 was hidden, mainly at least, in this particular group of diseases; and second, that, after its

[326] emergence as a distinct disease from the mass of other diseases characterized by continued fever, it was for a long time confused with them to such a degree that the mortality rate for typhoid fever obtained from the official figures is probably much lower than the true rate until about 1890.

It is very possible that typhoid fever was not infrequently confused with dysentery and diarrhœa and with "inflammation of the bowels." As late as the first half of the 19th century, dysentery was thought by many physicians to be a form of malaria, with which it, in common with many other diseases, was so often complicated. Few of the population, in certain sections of the City at least, escaped malarial fever at this time. It is not unlikely, therefore, that cases of atypical dysentery, especially when complicated with malaria, were mistaken for typhoid fever. "Inflammation of the bowels," from 1840 to 1897, and diarrhœa, from 1875 to 1900, were credited with a considerable number of deaths, and it is probable that these two more or less indefinite affections were confused with typhoid fever. Just to what degree the confusion with typhoid fever of deaths classified under these three categories affected the typhoid rate, it is impossible to determine.

On the other hand, it would be grossly erroneous to assume that all the deaths officially ascribed to typhoid were actually due to this disease. There are ample reasons for concluding that many deaths from pneumonia, pleurisy, empyema, influenza, appendicitis, suppurative processes of the liver and the biliary and urinary tracts, acute miliary tuberculosis, acute phthisis, and the various cryptogenic suppurative infections, as well as pyæmia and septicæmia, following wounds and injuries, including those caused by surgical procedures—the large group of affections often characterized by the "typhoid state"—were attributed to typhoid fever. Before the isolation of para-typhoid fever as a distinct disease, deaths from this affection were probably classed under typhoid fever, as they are even now under the international classification of causes of death.

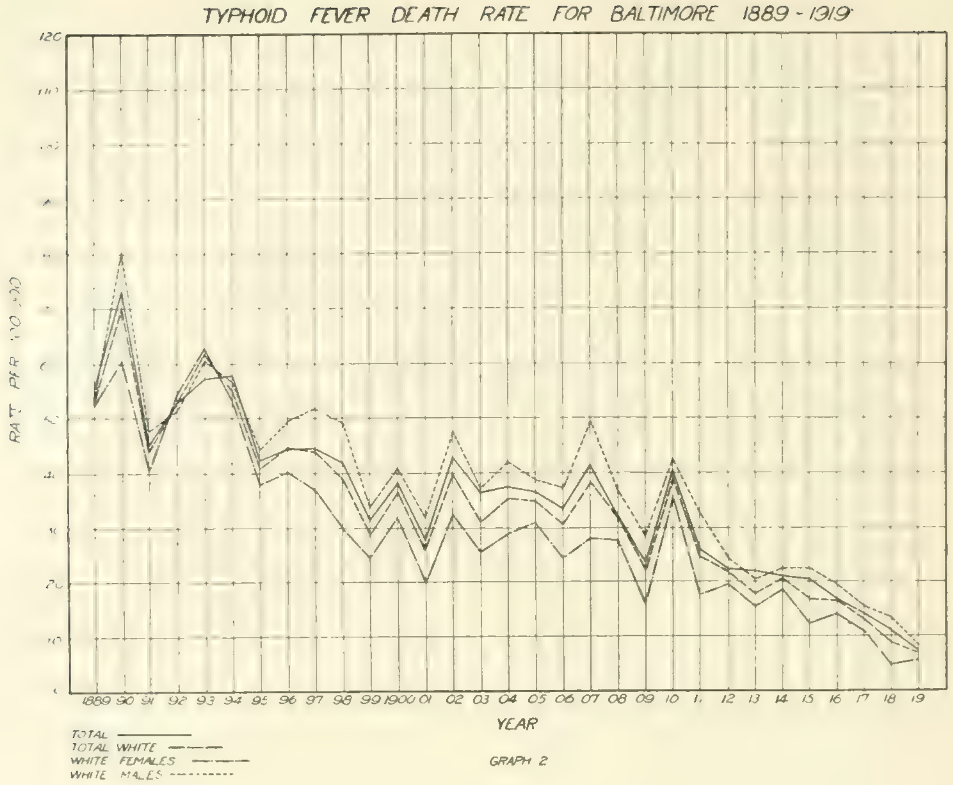
Another source of error to be taken into consideration is the influence upon the death rate of typhoid fever of deaths of non-residents, particularly those occurring among cases [326] brought to hospitals in Baltimore for treatment. No separate account of these was kept until 1917. In the three year period, 1917 to 1919, these deaths accounted for 20 per cent of the total deaths from typhoid fever in the City. The deaths within the City of non-residents from other States more than [327] counterbalance the few deaths from typhoid fever in Baltimoreans away from home. It is probably warrantable to assume that this source of error was of relatively small importance until transportation was made easier by improved roads and the use of the automobile from about 1905. Since this period, probably from 15 to 20 per cent of the deaths recorded from typhoid fever in Baltimore occurred among cases imported from rural Maryland for treatment. As early as 1890, the Baltimore hospitals received many cases of typhoid fever from Sparrows Point, and it must be recalled that certain thickly settled parts of the adjacent suburbs, Highlandtown for instance, were as readily accessible to some of the hospitals as many parts of the City.

From all the evidence at hand, therefore, it seems probable that the *shape* of the true mortality curve for typhoid fever for Baltimore, between 1851 and 1919, if we had the data for calculating it, would at least closely approximate that derived from the official figures, although the rates would be much higher in the earlier years.

White Population.—Graph 2 shows that the total rate for the white population exceeds the rate for the whole population only in 1893 and 1896. It closely approximates it in 1897, 1908, 1910, and 1916.

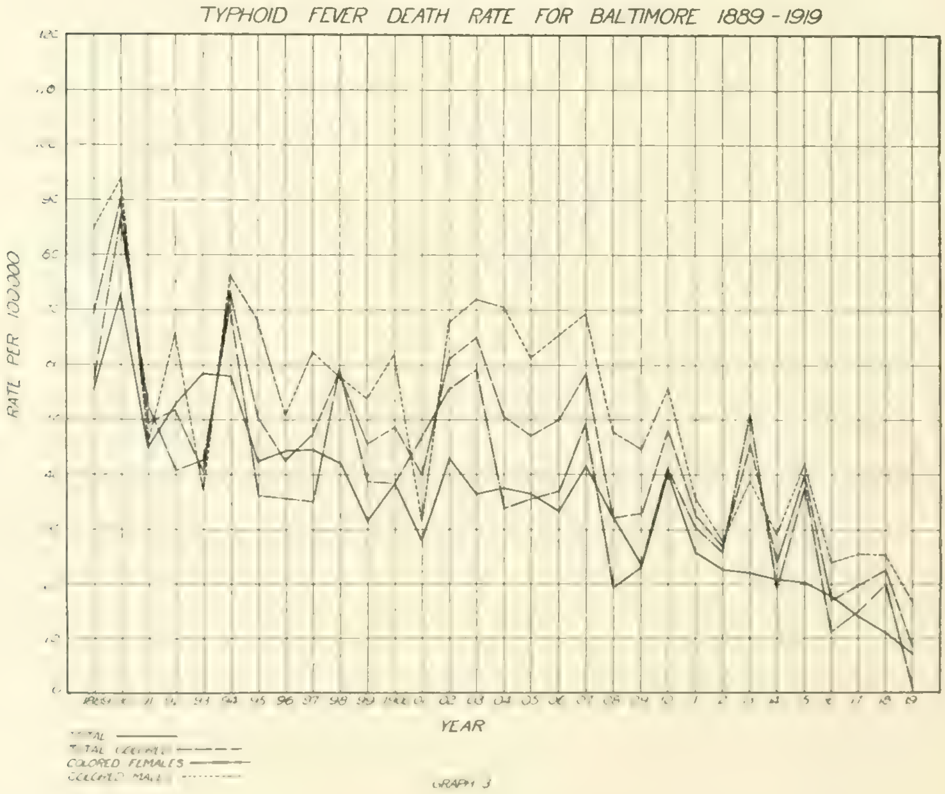
The rate for white females is well under the rate for the males, as well as under that for the total population, but it exceeds the rate for the total population in 1893. In general, the rate for the females follows accurately the rise and fall in the other rates, particularly the rate for total white and total population. In some few years, it falls or rises more abruptly than the other rates.

The rate for white males alone is uniformly higher than the other rates, with the following exceptions; in 1892, when it is slightly lower than the rest, and in 1894 and 1913, when it



TYPHOID FEVER DEATH RATE FOR BALTIMORE 1889-1919

GRAPH 2



TYPHOID FEVER DEATH RATE FOR BALTIMORE 1889-1919

GRAPH 3

is slightly lower than the rate for the total population. This [327] rate rises and falls uniformly with the other rates and the curves approximately fit each other.

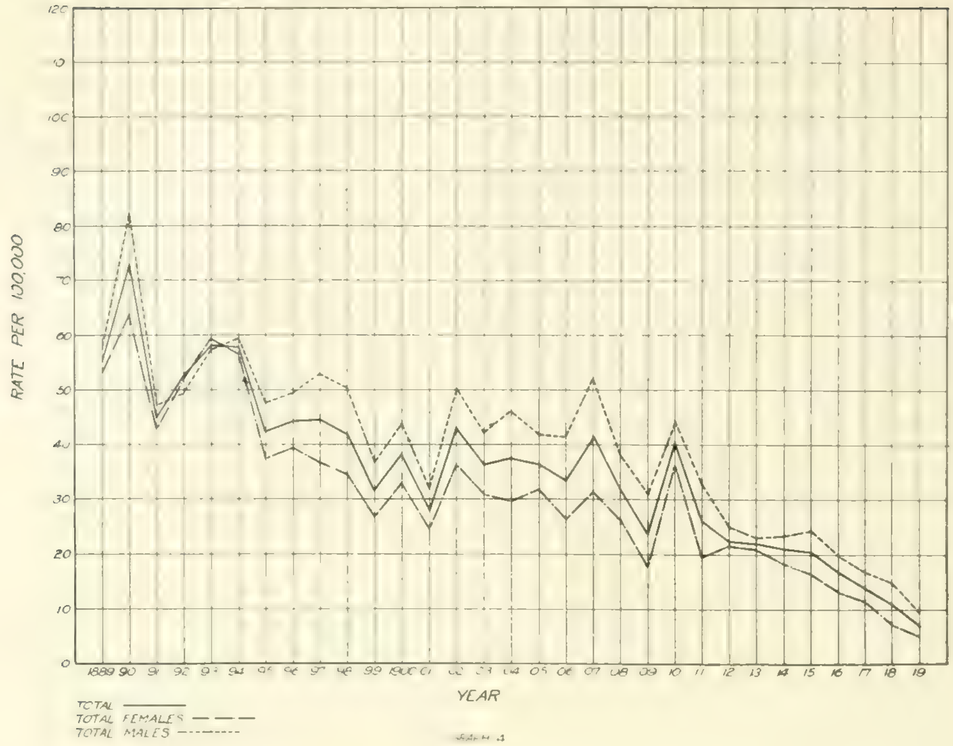
Colored Population.—The curves for the colored population in Graph 3 are much more complicated than those for the white. That for total colored falls considerably below the total rate in 1893, the year in which the rate for the total white population was above the total rate. The course of the curve for the total colored population in relation to that for the total rate is in the main quite different, in that it often fails to follow the dips and elevations in the total rate, but even when it does follow these, it is apt not to conform closely. In 1913, the year in which the total white rate dips considerably below the rate for the total population, the total colored rate, on the contrary, shows a marked peak. In 1914, when there is but a very small fall in the total rate, there is a considerable drop in the total colored rate. In 1915, this is compensated for by a considerable peak in the colored rate. In 1916, the colored rate falls slightly below the rate for the total population. In 1917 and 1918, there is again a decided rise in the total colored rate, with a marked drop in 1919. The highest rate for the whole series of years is the colored rate for 1890 which reaches 90.13 per 100,000 while the highest rate for total whites is 69.93 per 100,000 in the same year. As would be expected from the different proportions which they hold in the population, the curve for the negro rate is nothing like so uniform as that for the white rate.

The rate for colored males is uniformly higher than that for colored females, except in 1893, 1901, and 1913, when the latter is markedly higher. The peaks and depressions of the two curves for colored males and females generally follow each other. There are, however, some notable exceptions.

The curve for female colored is very irregular, but in general, it is higher than that for the total population. It is lower than the latter in 1895, 1896, 1897, 1904, 1908, 1909, 1914, 1916, and 1919. In some of these years, it is very noticeably lower.

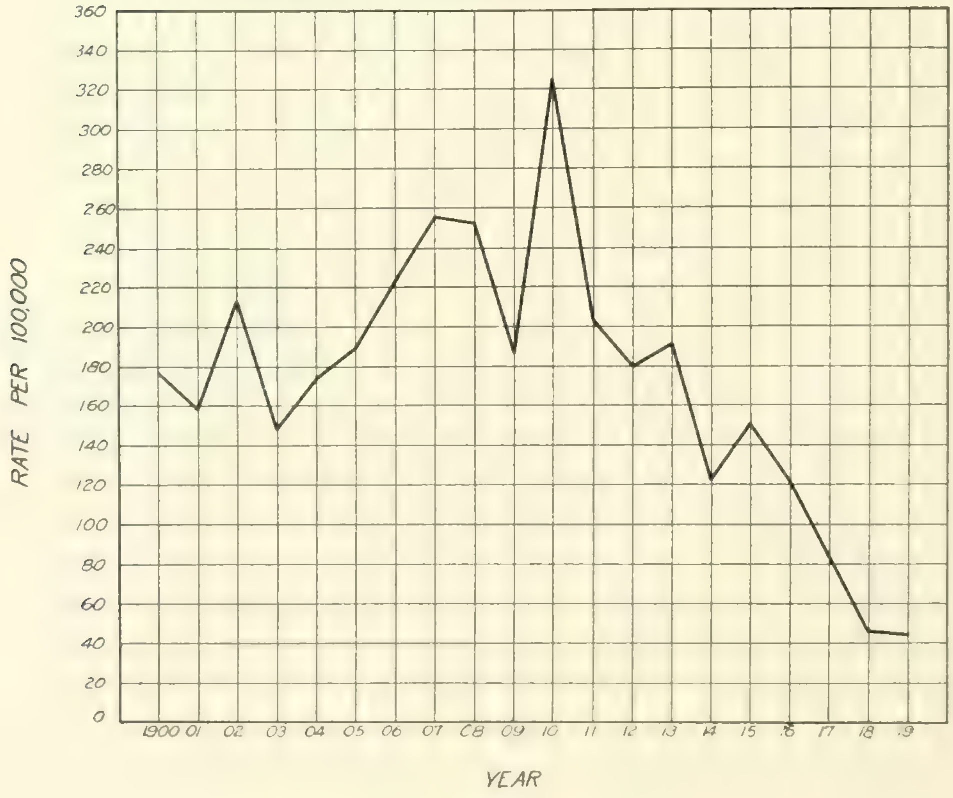
Males and Females.—There is remarkable uniformity in [328] the three curves plotted on Graph 4 which fit much more

TYPHOID FEVER DEATH RATE FOR BALTIMORE 1889-1919



GRAPH 4

TYPHOID FEVER MORBIDITY RATE FOR BALTIMORE



GRAPH 5

closely throughout than the curves previously considered. [328] The rate for females is slightly higher than that for both the total population and for total males in 1893, but with this exception, it is uniformly lower, and generally considerably lower than the rate for males. The rates for the two sexes vary as much as 20 per 100,000.

These graphs show very clearly throughout the period under consideration, from 1889 to 1919, inclusive, that the total rate has been markedly influenced by both sex and color, that the presence of negroes tends to raise the general rate, and that the presence of females tends to lower it. In other words, since the rates for the colored population are much higher than those for the white, and the rates for females of both races are lower than those for males, the total rate is influenced favorably by a low proportion of negroes and a high proportion of females in the population.

II. MORBIDITY

Although typhoid fever was by law made reportable in 1894, it was not until 1900 that the cases were reported with reasonable accuracy. In Graph 5 are given the morbidity rates per 100,000 inhabitants from 1900 to 1919, inclusive. It will be noted that the morbidity curve corresponds in a general way with the mortality curve throughout its course, and that from 1910 to 1919 the two curves fit with a fair degree

TABLE 8

TYPHOID MORBIDITY RATES PER 100,000 BY RACE AND SEX FOR 1918 AND 1919

Year	Baltimore							Rural Maryland		
	White males	White females	Total white	Colored males	Colored females	Total colored	Total morbidity	Total white	Total colored	Total morbidity
1918	61.32	33.71	47.05	29.44	47.51	39.11	45.82	......	......	......
1919	50.89	41.09	45.83	41.34	25.23	35.62	43.79	......	......	......
Average for 1918 and 1919.	56.10	37.40	46.44	35.39	36.37	37.37	44.81	152.24	262.88	172.85

[328] of accuracy. Unfortunately, data for comparing the morbidity in the colored and the white races are at hand for only 1918 and 1919. Comparison for these two years by sex and color is shown in Table 8. It will be noted that the morbidity rate for the colored is decidedly lower than that for the white population, just the opposite to that which obtains for rural Maryland, as is shown by the figures calculated from data kindly furnished me by Dr. John S. Fulton, Secretary of the State Board of Health. The difference in the ratio of incidence of the disease in the two races in Baltimore as compared with rural Maryland is, to some extent, explained by the larger proportion of imported cases and of out of town infections among the white population in Baltimore. So far as may be judged by the figures for these two years, the incidence of the disease is not only greater in the white race than in the colored, but is greatest in white males, and greater in white females than in either colored males or females.

III. Case Fatality Rate

Much interest attaches to the case fatality rate of typhoid fever in Baltimore, among other reasons because it has been used as a measure in estimating the degree of completeness of case reporting. Any conclusion in regard to case fatality must depend upon the accuracy with which cases and deaths are reported.

In his early studies on typhoid fever, Dr. C. Hampson Jones expressed the opinion, based upon the assumption that 10 per
[329] cent is a normal average case fatality in this disease, that the number of reported cases fell far short of the actual number existing—in some years by nearly 70 per cent. That the reporting has not been perfect is beyond question.

There are several fallacies not uncommon in studies of this character which should be pointed out. In the first place, it is difficult to collect the information on which to set a correct standard for the case fatality rate for any disease, and this is especially difficult for typhoid fever. One must have a large number of cases in which the diagnosis is established with accuracy by the use of refined laboratory methods, and the chance of burying under typhoid fever individuals dead of other diseases must be eliminated by submitting to autopsy [329] all the fatal cases in the series. An important source of possible error in Baltimore is due to the fact that many of the deaths registered as due to typhoid fever occur, as has already been pointed out, among cases brought from the counties of Maryland for treatment in the city hospitals, the reported cases being credited to the place of origin by the State Board of Health and the deaths then being recorded against Baltimore; *i. e.,* cases are transferred, but the deaths are not. The error lies in the fact that the divisor is disproportionately small. It is obvious, therefore, that for the purpose of establishing even an approximately correct case fatality rate, these two groups of cases and deaths must be reckoned, either combined or separately; *i. e.,* the county cases must be added to the divisor or the deaths among them must be subtracted from the dividend. The importance of this is well illustrated in Table 9, from which it will be seen that the average case fatality rate for the three years, 1917 to 1919, inclusive, cal-

TABLE 9

CASE FATALITY RATES

Year	I			II			III			IV		
				Baltimore and rural Maryland total cases and deaths			Rural Maryland			Baltimore		
	Baltimore cases	Baltimore and rural Maryland deaths	Percentage case fatality	Cases	Deaths	Percentage case fatality	Cases	Deaths	Percentage case fatality	Cases	Deaths	Percentage case fatality
1917........	544	92	16.91	696	92	13.22	152	24	15.79	544	68	12.50
1918........	302	73	24.17	396	73	18.43	94	14	14.89	302	59	19.54
1919........	293	49	16.72	327	49	14.98	34	5	14.71	293	44	15.02
Total and average.	1139	214	18.79	1419	214	15.08	280	43	15.36	1139	171	15.01

I. Official figures and case fatality rates.
II. Official figures and case fatality rates as corrected by addition of cases from rural Maryland.
III. Figures and rates for rural Maryland cases treated in Baltimore, and deaths among such cases.
IV. Correct figures and case fatality rates for cases and deaths properly chargeable against Baltimore.

[329] culated without making this allowance, is 18.79, per cent, while a much lower rate is obtained either by adding the total cases reported from rural Maryland to the divisor, or by subtracting the deaths among them from the dividend. It will be noted that the average for the three years for the cases and deaths occurring among Baltimoreans in Baltimore is 15.01 per cent. From Sections I, II, and IV of this Table, it is clear that the official annual and average annual case fatality rates for 1917, 1918, and 1919 (for the old twenty-four wards, as shown in Section I) are far from the correct rates. The source of error, of course, in Section I lies in the smallness of the divisor, from which is officially excluded the number of cases from rural Maryland, in proportion to the dividend in which the deaths occurring among these cases are included. In Section II, this error is removed by adding the cases in question to the divisor. Section IV gives the corrected rates for Baltimore based on actual cases and deaths reported, but excluding from the dividend the deaths occurring among the cases charged officially against rural Maryland. In Section III are given the correct rates for the cases from rural Maryland treated in Baltimore during the years in question. It will be noted that the average annual case fatality rates for Sections III and IV (*i. e.,* rural Maryland and Baltimore) are approximately the same.

It is evident that the annual case fatality rates derived from the deaths and reported cases officially charged against Baltimore are not the true rates. The method of deriving annual case fatality rates by dividing the number of cases into the number of deaths reported in a given calendar year is open to an error which may be considerable; not all the deaths that may occur among the cases reported in a given calendar year take place within that period of time. The early months of the calendar year will usually have deaths occurring among cases reported during the late months of the previous year, and this process may be expected to continue throughout the series of years under consideration, and the last year in the series will almost surely lack some deaths. This error may be reduced to some degree by averaging the rates for a series of years.

A more accurate method is to take into account only the deaths occurring within the group of cases, allowing a reasonable length of time for all the fatalities likely to happen within the group. This was done for the cases reported within the old twenty-four wards during 1919. [329] In the 293 such cases there occurred 44 deaths—41 during 1919 and 3 between January 1 and May 1, 1920. [330] The case fatality rate thus derived, 15.01 per cent, happens to be the same for this single year as that for the average for the three years, as calculated by the usual method.

TABLE 10

Case Fatality Rates, According to Sex and Color, 1919

Baltimore, 1919, twenty-four wards

	Mortality	Morbidity	Percentage case fatality
Total white	35	259	13.89
Total colored	9	34	26.47
Total males	27	159	16.98
Total females	17	134	12.68
Male white	20	139	14.38
Female white	15	120	12.50
Male colored	7	20	35.00
Female colored	2	14	14.28
Total white and colored	44	293	15.01

The accurate data for determining the influence of sex and color upon the typhoid case fatality rate in Baltimore are rather meagre, being limited to the year 1919. These are set forth in Table 10. On account of the smallness of the figures for some of the categories and the absence of correction for age, some of the percentages are perhaps subject to considerable probable error. Taking them at their face value, however, the risk rate of dying of clinically recognized typhoid fever is twice as great for colored as for white, somewhat greater for white males than for white females, and twice as great for colored males as for colored females, and nearly a fourth greater for total males than for total females.

SUMMARY OF THE KNOWLEDGE OF THE INFLUENCE OF DIFFERENT FACTORS ON TYPHOID FEVER IN BALTIMORE

From the mass of facts set forth in the preceding pages, it is evident that most of the various factors concerned in the spread of typhoid fever have acted continuously, but with varying degrees of force, during the whole period. The most important of these factors are:

(1) Water Supply.
(2) Crude Methods of Sewerage Disposal.
(3) Milk.
(4) Importation of cases and carriers in:
  a. Immigrants.
  b. Military.
  c. Isolated Individuals.
(5) Infection contracted without the City.
(6) Associative Contact with Recent Cases and with Remote Cases; *i. e.,* Carriers.
(7) Flies.
(8) Bathing in Polluted Waters.

Water Supply.—The water supply was seriously polluted from the earliest times until 1911, but the degree of polluhas varied at different periods and with the sources from which the water was obtained. In 1850, at least a half and probably two-thirds of the drinking water was obtained from wells within the City. The proportion obtained from these sources was notably reduced after the City took over the water supply from the private company and provided a more abundant and better distributed supply after the establishment of the Lake Roland Dam on Jones' Falls in 1862. Nevertheless, a large part of the inhabitants clung to the internal water supply from springs and wells until long after this date. Through the efforts of the Health Department, these were practically eliminated in the old City of that period by the time of the annexation in 1888. It is probable that the Lake Roland watershed became heavily infected with typhoid fever during the Civil War. The completion of the Gunpowder water supply system, with its numerous storage reservoirs, and the substitution to a very great extent of water from this source for the old Lake Roland water supply must have favorably influenced the course of typhoid fever for a considerable period after 1881. Indeed, with the exception of a few years after the annexation of 1888, the whole trend of the typhoid fever curve was distinctly downward for the next twenty years. After 1901, the water supply again became more and more polluted, and there was an upward wave in the typhoid fever curve until chlorinization of the water supply was introduced in 1911, after which there was a distinct drop. As has been pointed out, the water supply was never obtained from a single source until 1915. The advantages which should have accrued from the various changes in the water supply were never wholly reaped because the old conditions were but slowly replaced, the older and poorer supplies being never entirely abandoned. For instance, after the introduction of the Lake Roland supply, a large proportion of the population still clung to the old springs and wells, and after many of these were abandoned in the old City in consequence of the activities of the Health Department, new sections which derived their water supply entirely from springs and wells were taken into the City; then too, after the introduction of the Gunpowder water supply, the old Lake Roland water supply was still to a great degree mixed with it. There is reason to think that the Lake Roland water supply obtained from Jones' Falls had become seriously polluted before the greater number of wells and springs were closed and this water was substituted. While it probably prevented a marked increase in the death rate, it exerted no great influence on its actual reduction from its previous level. It would appear that an infected external was substituted for an infected internal water supply. [330]

Sewerage.—It has been shown that until 1915, with the exception of a few private sewers leading by the streams into the Basin and Harbor, human excreta containing typhoid bacilli from the cases and carriers emitting them were deposited in or on the ground. Had all the water supply been extra-urban and had all the dejecta gone into the soil, amateur hygienists to the contrary notwithstanding, there could be no serious objections to this method on sanitary grounds. But

[330] the water supply, until a late date, was largely of intra-urban origin, and the City was very closely built up. However, the spaces intended for drainage in many if not most cesspools became stopped, and the latter in consequence took on the attributes of surface privies. At one time it was the fashion to build water-tight cesspools, and the contents of many of these overflowed into streets, alleys, courts, and cellars. Material from many of the cesspools and privies, therefore, reached the wells and springs from the surface as well as through the soil. This material from overflowing cesspools and privies, as well as from the night-soil dumps, scows, and carts, was readily accessible to flies, the breeding and feeding of which in swarms were favored by the conditions of the stables, the imperfect surface drainage, and the inefficient collection and disposal of garbage. The methods of disposal of human waste favored,
[331] to a high degree, the spread of typhoid fever by water, flies, and by milk and other foods. With the development and the perfection of the extra-urban and the gradual abandonment of the intra-urban water supply, the cesspool and the surface privy lost much of their importance as sources of typhoid infection. It is evident, that if the wells and springs had been abandoned sooner, the privy and the cesspool, though still harborers, would early have lost in importance as distributors of typhoid infection. After 1890, infection from this source was practically confined to that conveyed by means of flies. It is clear, therefore, that so far as concerns action other than from sources of infection for intra-urban drinking water, and the milk, and perhaps other foods, the sewerage remained a rather constant and relatively unimportant factor in the spread of typhoid until the new system was brought into general use in 1915. After 1915, it was possible for the sewerage system to exert in this direction but comparatively little influence on typhoid fever.

Milk.—During the whole period between 1851 and 1894, conditions were most favorable for the spread of typhoid fever by means of milk. In the first place, much of it was from cows kept within the City or nearby suburbs, where every chance existed for its pollution from actual cases of typhoid fever and from carriers. It was heavily watered, for much of the time with water from polluted wells and springs. It is [331] not improbable that the substitution of less polluted water for watering milk before 1894 and the interference by the Health Department with watering after this date affected the typhoid death rate favorably. Under the laws of 1896 and 1902, the Health Department was able to improve very markedly the conditions under which milk was produced and sold within the City and thus to cut down very materially the opportunities for the dissemination of typhoid fever by milk produced and infected within the City. Under these laws, the urban cow was to a great extent but not entirely driven out. While these laws did not forbid the keeping of dairy cows within the City, they made it impossible to keep them within the closely built up sections and, on the whole, unprofitable, on account of the expense of constructing stables of the type required to keep them at all except in certain outlying districts. A great revolution in the control of the milk supply followed the enforcement of the milk ordinance of 1908, and by 1912 considerable oversight and control had been gained by the Health Department over the milk supply on dairy farms as well as within the City itself. Still between 1912 and 1918, numerous larger and smaller milk outbreaks were traced by the Health Department. As late as 1917, nearly 8 per cent of the reported cases were traced to milk in readily recognizable outbreaks. Besides these, there must have been large numbers of isolated or small groups of cases, in which the infection was carried by milk and which the machinery of the Department was inadequate to detect. The regulations of the Health Department could not, of course, prevent the removal of empty milk bottles from households with typhoid fever patients until such cases had been reported, and under the most favorable conditions two or three weeks pass between the beginning of the illness and the report of the case. Unless milk bottles are uniformly sterilized as a matter of routine, bottled milk may be, during the prevalence of typhoid, more dangerous than milk served from the old-fashioned churn. When milk is sold from the churn or can, the purchaser receives it in his own container, and the dairy takes nothing infected away from the householder. The compulsory sale of milk in bottles with-

[331] out adequate provision for the cleaning and sterilization of the latter is a sanitary reform of doubtful value from certain standpoints. Until 1918, the refrigeration of the milk supply, whether in churns, cans, or bottles, was, with certain notable exceptions, quite inadequate, and in warm weather conditions were favorable for the multiplication of typhoid bacilli that reached milk.

It is only since the enforcement of the milk ordinance of 1917 that there has been reasonable security against milk-borne (including ice cream) typhoid in Baltimore. This law requires not only pasteurization and refrigeration of the general milk supply, but the cleansing and sterilization of all milk utensils, including the farmers' cans. No cases of typhoid fever were traced to milk in 1918 and 1919. It must be borne in mind, however, that owing to the great diminution in the number of cases of the disease in rural Maryland, as well as within the City, the opportunities for the infection of milk have become correspondingly less.

Importation of Cases and Carriers: (*a*) *Immigrants.*—It is well established by the writings of Nathan Smith and Bartlett, that typhoid fever was prevalent in New England before 1826 and was well established there as the most common continued fever by 1842. Some years later, Bartlett showed that the disease was widely diffused throughout this country, south as well as west.

From 1800 to 1850, Baltimore grew very rapidly by immigration; the chief sources of immigration from abroad being England, Scotland, Ireland, and Germany. There was during this time, especially in the earlier years, a large immigration from New England and Pennsylvania, as well as from Virginia. In all of these places typhoid fever was then widely prevalent, and it is therefore certain that these immigrants as clinical cases and carriers contributed largely to the spread of the disease, doubtless long before strongly entrenched here. The later European immigrations were all from typhoid stricken countries. Baltimore's great prominence as a port city added to this danger. In the lower part of the City, where recent immigrants mostly settled or lodged, while transients, conditions were especially favorable for contamination of the

wells and springs and for the spread of the infective agent by [331]
contact and by flies.

Importation of Cases and Carriers: (*b*) *Military.*—For the ten-year period before the Civil War, the average mortality rate for typhoid fever in Baltimore was about 45 per 100,000. From 47.09 in 1860 it jumped to 75.92 in 1861, remaining high during the whole war period and for some years afterwards. The highest portion of the typhoid mortality curve lies between 1861 and 1872. During the whole war, Baltimore and its vicinity were crowded with federal troops brought
from widely separated parts of the country and from the [332]
hospitals and battle-fields of Virginia, West Virginia, and probably Kentucky, and perhaps further south and west. The City became a great collecting and distributing point for troops. There were a number of military hospitals, some very large, for the treatment not only of the wounded, but of the sick. All the facts favor the inference that the presence in the City of great numbers of transient soldiers of an army known to be highly affected with typhoid fever must have resulted in an increase in the morbidity and mortality rates of this disease among the inhabitants. It is very probable that the soldiers were an important factor in spreading infection, not only through the wells and springs within the City, but over the Lake Roland watershed.

Importation of Cases and Carriers: (*c*) *Isolated Individuals.*—In addition to the large immigrations, attention should be called to the fact that large numbers of cases were annually brought into the City for hospital treatment, and even of late years a not inconsiderable proportion of the typhoid fever cases and deaths have occurred among individuals, from other states and on foreign ships, who have arrived in the City with the disease.

It has been pointed out above that for the three years just past, 20 per cent of the total deaths of typhoid fever in Baltimore occurred among individuals brought in to Baltimore hospitals from rural Maryland for treatment. On the whole, it seems probable that the mortality rate for typhoid fever in Baltimore has been, throughout the history of this disease,

[332] materially influenced by the importation of isolated cases of the disease.

Infection Contracted Without the City.—As far back as 1890, it was recognized in the Health Department that a considerable proportion of the cases of typhoid fever reported in Baltimore was contracted without the city limits, either in rural Maryland or in other states. It has been shown that in 1919 over 12 per cent of the reported cases certainly received their infection outside of the City and that 8 per cent probably had this source of origin. Until the last few years, there is reason to think that many cases were infected at resorts on the Bay and the numerous small rivers and estuaries of the Bay, near the City, particularly at the so called " shores." When the great prevalence of typhoid fever, until recent years, in rural districts of Maryland and other states, which Baltimoreans visited for work or pleasure, is taken into consideration, it seems probable that in the past this source of infection was even more important than at present.

In this connection, it is significant that the morbidity rate for Baltimore has declined in parallel with that of rural Maryland, particularly in the counties adjacent to Baltimore, in which the greater proportion of infection took place, and that nearly all of the cases of typhoid fever, attributed to these sources of infection within the last few years, have occurred in the warm weather when typhoid fever is most prevalent in the country districts.

Associative Contact with Recent Cases and with Remote Cases; i. e., Carriers.—If 12½ per cent of the cases could be traced to contact with recent cases in households and hospitals and nearly 8 per cent to contact with remote cases in households, *i. e., carriers,* in 1918 and 1919, it is reasonable to suppose that these factors exerted at least as great an influence upon the people in former years, since their conditions of life and their habits were much more favorable for infection by associative contact than at present.

Flies.—Enough has been said under other headings to indicate that until very recently the general sanitary conditions of the City were peculiarly adapted to fly-borne typhoid. It remains to be pointed out that, particularly in the crowded sections of the City and among foreigners, the great numbers [332] of flies had ready access to the excreta of typhoid patients. The fly season is a long one, screening was almost unknown, and among certain elements in the population, the screening of the apartments of typhoid fever patients was enforced with great difficulty or not at all.

Bathing in Polluted Waters.—It is probable that until the last few years, this has been a frequent source of infection of typhoid fever within the city limits. It is not improbable that many persons were infected by bathing in the polluted streams within the city limits, as well as in the Basin and in the middle branch of the Patapsco at Spring Gardens.

UNEXPLAINED CASES

The question of the source of infection of the group of *unexplained cases* must be faced. Even if it be granted that the explanations offered for the *explained cases* are adequate—and the value of some of these (probable and possible out-of-town infections, infection from remote cases in which carriers were not demonstrated by culture methods, and neighborhood infection by flies) is acknowledged to be questionable—there still remains a large group of cases upon the origin of which no light has been thrown.

In the absence of some other obvious explanations, it is natural and usual to attribute these cases to water and milk-borne infection. But slight consideration shows this to be unsatisfactory, and the same question arises in all cities with comparatively low and falling typhoid fever rates, following the inauguration of apparently effective water supply and sewerage and milk protection systems. In the old twenty-four wards of Baltimore, the sewerage system—with the exception of certain districts which, however, do not show any particular difference in typhoid incidence—may be eliminated from consideration. If any dependence can be placed upon the value of the bacteriological examinations conducted independently in the laboratories of the filtration plant and of the Health Department during 1919, the city water supply in that year at least, according to the accepted and apparently logical standards, may be acquitted. The number of reported

[332] cases on the watershed were very few and were supervised both by the Water Department and by the very efficient deputy State Health Officer. If the water supply were responsible for some or even most of the cases now under consideration, it is reasonable to suppose that there should have been an even greater number than actually occurred.

[333] To state that no cases were traced to milk is not equivalent to asserting that infection was not spread through this medium. It is unwarrantable to assume that all chances of the spread of the disease by milk were successfully guarded against. No one concerned with the oversight of the milk supply thinks that all the milk utensils were sterilized and that all the milk was adequately pasteurized. There is still a chance that some typhoid fever in Baltimore is spread by milk. However, if the milk supply was even an important source of infection for this group of cases, explanation would still be lacking for the comparatively large proportion of these cases in which the use of milk as a beverage was denied.

When this *unexplained* group is analyzed, it is found that the cases do not vary essentially from the *explained* group in age, sex, color, or seasonal and locality distribution. They vary in only one attribute.

It is certain that, in some cases at least, more complete histories could have been elicited. Some of the patients were not only illiterate and stupid, but many could not express themselves in languages familiar to the investigators. It must also be recalled that many of the patients were too ill at the time of the investigations to give clear answers and to recall all the details of their histories. In such cases, the investigators had to rely upon the testimony of relatives and friends. Again, it is not possible for an individual to recall all his actions and contacts some weeks after an event. After all, the modes of transmission inquired into and which we have been considering are only the very obvious and perhaps there are others quite as important.

There are in this community hundreds of survivors of clinically recognized and unrecognized attacks of typhoid fever who are potentially carriers and who from time to time may pass on the infective agent to others. A colored cook, proven to be a carrier responsible for 9 cases of typhoid fever among [333] college girls and who appeared to be quite frank when submitted to the closest interrogation, was unable to recall a febrile illness suggestive of ordinary clinical typhoid fever. She described, however, very clearly the symptoms of an attack of acute cholecystitis which she had had about a year before. Her story was confirmed later, even as to dates, by her physician. She apparently had had an attack of primary cholecystitis due to *B. typhosus* and resulting in the carrier state. Had she not by chance transferred typhoid infection to a number of college girls at the same time, she would probably not have been apprehended. Upon such chances as these the transmission of this disease and the recognition of its mode often depend. None of this woman's personal associates gave a history of frank typhoid fever either before or after her attack of cholecystitis. The victim of typhoid fever rarely knows the typhoid fever history of his intimate associates outside of his family, and, when questioned, cannot tell whether any of his hosts or hostesses or their servants have had the disease. To find a hidden carrier, it would be necessary to take a census of the patient's known and unknown contacts. A large proportion of the several hundred food handlers whom I have had cultured with negative results may be intermittent carriers, infecting from time to time people not intimately associated with them. There must be in this community a considerable number of so called "healthy carriers," typhoid carriers who have not developed clinical typhoid fever and who are effective infectors, or who may become self-infected; *i. e.,* their barriers of resistance may at some time become ineffective against typhoid bacilli on the surface of their bodies (on the mucosæ of the biliary, intestinal, or urinary tracts). It is impossible to set a time limit between the beginning of the healthy carrier state and true infection.

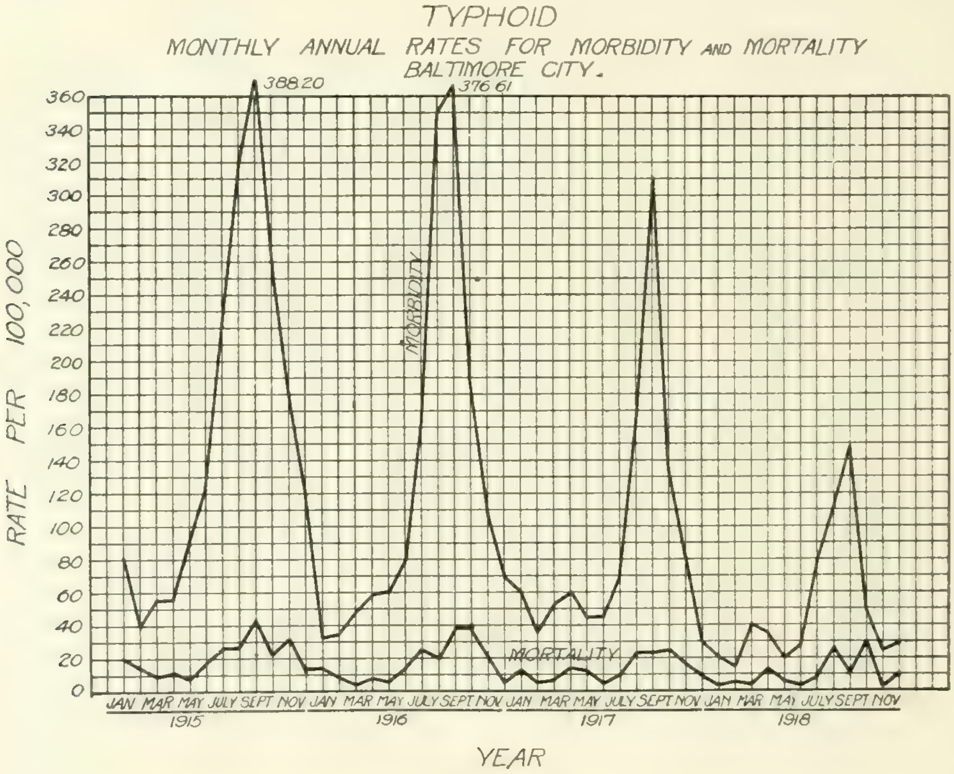
If butter and certain kinds of cheese were exhaustively studied, with more refined methods than now exist for isolating *B. typhosus,* it is probable that it would be found that these foods are a not uncommon means for the transmission of typhoid fever in urban communities.

Now that the City Fathers, after many years of fumbling,

[333] have finally made those general sanitary provisions necessary for the reduction of the incidence of typhoid fever to a comparatively low level, in the old twenty-four wards, and the obvious may be set to one side, the public health administrator of an inquiring turn of mind may turn himself to the much more interesting and important problem of determining the less obvious or hidden means of transmission of the disease.

SEASONAL DISTRIBUTION

It has long been held that typhoid fever in Baltimore is essentially a disease of the summer and autumn. This has not always been the case, for during many years, from 1856 to 1881, the number of deaths in the winter months—December, January, and February—equalled or even exceeded those for the summer and fall months. It is interesting that this was the period during which so much of the water supply was intra-urban in origin and when the extra-urban supply was entirely or mainly derived from Jones' Falls. Since 1881, the highest monthly mortality has remained permanently in the summer and fall months. During this period, the springs



GRAPH 6

and wells in the City as a whole were abandoned and the Gunpowder water supply was largely or entirely substituted. As long as the water supply was grossly and steadily polluted, the distribution of the disease tended to be more evenly distributed throughout the year. [334]

A study of the reported cases since 1900 shows that from February, the month of lowest incidence, there is a very gradual progression of the disease until July when there is a rapid rise, culminating in a peak usually about the middle of September and more rarely late in August, or even early in October. The decline is usually somewhat abrupt during September or October and more gradual in November, December, and January. The deaths follow the same general course, lagging about one month behind the reported cases. This course of the disease is well shown on Graph 6, on which the monthly annual morbidity and mortality rates are given for the years 1915 to 1918, inclusive.

Analysis of the modes of action of the factors influencing the incidence of typhoid fever in Baltimore makes clear why, during the second period, the greatest incidence and mortality should be in the warm months. This is the season when the greatest number of imported cases occur, when conditions are most favorable for infection by means of milk, milk products, and other foods (and in which consequently most of the observed milk epidemics have occurred), when there are the greatest number of cases upon the watersheds, and when conditions are auspicious for the dissemination of the infective agent by carriers, clinical cases, flies, and bathing in polluted waters.

REFERENCES

William T. Howard, Sr.: Personal Notes on the Medical Lectures of John K. Mitchell, Professor of Medicine, Jefferson Medical College, 1842-1843. Notes on Cases observed at the Baltimore City and County Almshouse, 1843-1844. Conversations with the writer.

Elisha Bartlett: The History, Diagnosis, and Treatment of Typhoid and of Typhus Fever; with an Essay on the Diagnosis of Bilious Remittent and of Yellow Fever. Philadelphia, 1842. The History, Diagnosis, and Treatment of the Fevers of the United States. Philadelphia, 1847.

[334] Thomas H. Buckler: History of Epidemic Cholera as it appeared at the Baltimore City and County Almshouse in the Summer of 1849. Baltimore, 1851.

Thomas H. Buckler: The Basin and Federal Hill; Belvidere and the Line of Calvert Street. Baltimore, 1874.

James Wynne: Extract from the First Report of the Committee on Hygiene of the American Medical Association, 1850.

Sewerage Commissions of Baltimore: Reports of 1862, 1894, 1895, 1896, 1897, and 1905-1910.

Calvin W. Hendrick: Sewerage System; History of Baltimore, Clayton Coleman Hall, Editor, 1912, Vol. I, pp. 424-428.

Alfred M. Quick: Baltimore Water Works. *Ibid.*, pp. 413-423.

Reports of the Water Department of Baltimore of various years.

W. W. Ford: Some Observations on the Bacteriology of the Baltimore City Water. Johns Hopkins Hospital Bulletin, February, 1911, p. 53. The Problem of Typhoid Fever in Baltimore. *Ibid.*, October, 1911, p. 357.

Reports of the Department of Health of Baltimore, 1850-1918.

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THE REAL RISK-RATE OF DEATH TO MOTHERS FROM CAUSES CONNECTED WITH CHILDBIRTH.*

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INTRODUCTION.

It is a fair statement that few, if any, of those interested officially and unofficially in public health in the cities and states of this country have any really intelligent appreciation of the high fatality risks of pregnancy and of delivery from that state. Seriously thoughtful consideration of these subjects is almost entirely confined to a few leading obstetricians, whose warnings and teachings only too often fall upon deaf ears. With the exception of these and of some of the more intelligent of those engaged in the infant welfare movement who have realized from practical experience, supported at times by figures not often dignifiable by the term statistics, that the reduction of infant mortality is to a large degree bound up with pre-natal, natal, and post-natal care of the mother, few in this country have any accurate conception of the risk run by the average woman in her lying-in. The great movements directed against puerperal infection since Semelweiss' discovery, Holmes' crusade, the exposure of the deadly obstetrical hospitals, the recognition of the bacterial causation of the disease, and the perfection of the anti- and a-septic methods exhausted themselves, after improving obstetrical hospitals, teaching, and nursing, in inadequate regulations of midwives. In the meantime, researches in obstetrics advanced knowledge in regard to the recognition and control of other causes of maternal mortality in the pregnant and lying-in states; such as, hemorrhage, intoxications, malformations and malpositions, rupture of the uterus, and the like, which, taken together, account for the larger moiety of maternal deaths. But, the relatively highly developed knowledge concerning practical obstetrics is so meagerly diffused and so ineffectively applied to practice, that certainly in the registration area of the United States it is not brought to bear with sufficient force to prevent the occurrence of innumerable major and minor illnesses and a large number of unnecessary maternal deaths.

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One of the most important functions of vital statistics is to determine accurately the risk of illness and of death of those actually exposed to a particular disease or disease group, or, in other words, to measure specifically the forces of morbidity and of mortality. The terms of the risks so determined are commonly expressed in ratios or rates indicating the proportions of the numbers affected to the numbers exposed, and the real value of such rates must depend upon the accuracy and the comprehensiveness of the data in regard to both the affected and the exposed. It is only when the data include such essential qualities as age, sex, race stock, and occupation that the resulting rates may be regarded as satisfactorily specific. In actual experience, the data available to vital statisticians are, in general, so lacking in comprehensiveness in these respects, that there exists hardly a single example of an accurately defined rate expressed specifically in terms of risk on the part of those definitely exposed to illness or to death from a particular disease. In consequence, the public health official is seriously handicapped in defining his problems, in devising administrative measures, and in estimating their value. Likewise, the student of the natural history of disease must so often appeal in vain to the statistical method for accurate measurement of the forces of morbidity and mortality and must remain content with rates relatively crude and subject to various adjustments in the process of being rendered specific for even one or two categories. In measuring the force of mortality in affections of the puerperal state, it has been customary to use rates based on the number of maternal deaths in relation to (*a*) the whole population, (*b*) the whole number of females, (*c*) the number of females within the usual childbearing ages (15 to 45 years), or (*d*) the total number of labors as evidenced by the number of reported live births. Of these methods, the latter is obviously the only one that even approaches specificity; the others are, in the nature of things, necessarily so general and so wide of the mark that they are evidently useless for any serious attempt to estimate the force of mortality in a single community, or to compare it either in the same community over a term of years or in different communities. Risk-rates calculated on the number of maternal deaths from causes connected with the puerperal state and the number of live births occurring within a given period are subject to two important sources of error. The number of live births, used here as the divisor, does not accurately measure the actual number of labors, i.e., the number of women exposed to death in childbearing; for some labors produce more than one living child, and stillbirths, which in some cases are dependent upon conditions that materially enhance maternal risks and which represent a variable but not inconsiderable number of labors, are left out of account.

It occurred to me in 1917, when confronted with problems of this character in the Health Department of Baltimore, that by using the sum of the living and of the still births reported and the number of the deaths of mothers ascribed to puerperal affections, annual death rates might be obtained, expressing in specific terms the actual risk of dying for women definitely exposed in childbearing. Stated in formal terms, the true puerperal risk-rate in childbearing would be derived from the formula:

$$RP = \frac{DP}{BL + BS},$$

where DP equals the number of maternal deaths due to causes definitely connected with the puerperal state and BL and BS equal the number of live and still births respectively.

Risk rates calculated on this basis for the total deaths in the puerperal state and for the deaths under the different rubrics 134 to 141, inclusive, of the international classification of the causes of death, separately and in various combinations, on the data of the Baltimore Health Department for 1915 to 1917, gave interesting tables. Owing to inability to find data of sufficient value on which to base comparative studies—the Reports of the Registrar-General of England and Wales and the Birth Reports of the United States Census for the birth registration area being silent on stillbirths—the matter was dropped for the time. Recently, on becoming interested in the subject again, it occurred to me that by utilizing the data of several large American cities, in which it might be assumed that the reports of live and still births and the reporting and classification of deaths are reasonably accurate, figures sufficiently large to eliminate chance and probable errors might be obtained. For this purpose, New York, Philadelphia, Boston, and Baltimore were chosen for the five year period 1915 and 1919, and, as will be pointed out later, there is a remarkably close correspondence in the rates obtained, and the rates calculated from the figures of these cities seem to afford a fairly accurate criterion of the risk-rate of death applying to childbearing in women in these particular urban communities. Fortunately for purposes of comparison of the value of the method and investigation of the whole subject on a broader scale in this country, the report for 1918 of the Bureau of the Census on births within the registration area includes stillbirths, and this large material is now available for the first time for such uses.

Before discussing the results of this inquiry, it is necessary to point out that on looking further into the question, I found that in the idea of taking stillbirths into consideration I was anticipated by Dr. William Farr, at least. Deeply interested in this subject from a date early in his connection with the Registrar-General's Office (discussing this subject first in the Fifth Annual Report, 1843), he dealt with it fully in the Seventeenth Annual Report in 1856, when he utilized the data of the eight year period 1847 to 1854, which he expressed in tables of rates obtained by diving the material deaths by the total number of live born children. But he clearly recognized, that, as the term *Births,* as used in the returns, expressed only the number of children born alive and not the exact number of childbearings or accouchements, to ascertain the true danger of childbirth to mothers, it was necessary to take into account not only the reported live births, but stillbirths and plural births. Correction for plural births on the basis of the returns for 1852 indicated the necessity of a reduction of about 1 per cent. from the number of reported births, and correction (based on some French figures) for stillbirths required the addition of about 3 per cent; so it would appear that if something like 2.5 per cent. be added to the number of reported live births, the sum would indicate with some degree of accuracy the actual number of childbearings. He concluded that "on an average of eight years (1847 to 1854), to every 10,000 children born alive 54 mothers died; so about 53 in every 10,000 or 1 in 189 accouchments were fatal." Farr classed puerperal deaths under metria—the term adopted for puerperal infection in his statistical nosology—and all other causes. In the same article, he presented the following table, showing the mortality per 100,000 childbearings of women of different ages:

TABLE I.

Ages of women.	Total deaths.	Deaths by metria.	Deaths by other accidents.
15–25	668	277	391
25–35	425	148	277
35–45	633	154	479
45–55	883	163	720

Commenting on this table, Farr pointed out that the mortality in childbearing is least during the age period 25 to 35—when more than half of the children are borne by their mothers—and that as the rates of mortality in the last three age periods are in nearly the ascending ratio as 2, 3, and 4, the calamitous deaths of mothers in childbed are governed by a mathematical law. He further estimated that the death rates for women from all causes for these age periods were raised by childbearing risks per 100 as follows:

15–25	.820 to 1.488
25–35	.327 to 1.417
35–45	.535 to 1.831
45–55	.874 to 2.496

In the Thirty-third Annual Report in 1870, Farr, replying to the criticisms of Mathews Duncan, demonstrated that the returns to the Registrar-General were reasonably complete and that the figures used by him included deaths only in childbearing and excluded deaths due to other causes but occurring in pregnant or childbearing women; i.e., that the classification was designed to show the actual causes of death. Among other things, Farr showed that in eleven large English towns the childbearing mortality was 4.9 and in sixty-four healthy country districts, 4.3 per 1,000.

Returning again to the subject, in the Thirty-ninth Annual Report of 1876, Farr pointed out that in the thirty years between 1847 and 1876, 106,565 mothers died in England in childbirth, or about 5 in every 1,000 or 1 to every 200 children born alive. The proportions varied from year to year, the lowest per 10,000 live born children being 42 in 1857 and the highest, 69 in 1874. In 1876 it was 47.

TABLE II.

Maternal death rates 10,000 live births from causes connected with the puerperal state, in England and Wales, 1847–1876, together with the percentage contributed by each separate cause to the whole number of such deaths.

(Condensed from the table of Dr. William Farr, Thirty-ninth Annual Report of the Registrar-General, 1876, page 279.)

1847–1876, inclusive.	Living births.	Accidents of child birth.			Metria.			Metria and child birth.	
		Deaths.	Rate per 10,000 births.	Per cent. total metria and child birth.	Deaths.	Rate per 10,000 births.	Per cent. total metria and child birth.	Deaths.	Rate per 10,000 Births
Total....	21,292,995	68,241	32.05	64.04	38,324	18.00	35.96	106,565	50.05

TABLE III.

Maternal death rates per 10,000 live births from causes connected with the puerperal state, and the percentage contributed by each separate cause to the whole number of such deaths, in England and Wales, 1911–1915.

Year.	Living births.	134			135			136			138			139			140			141			Total puerperal state, excluding puerperal septicemia.			137			Total puerperal state.	
		Accidents of pregnancy.			Puerperal hemorrhage.			Other accidents of labor.			Puerperal albuminuria and convulsions.			Puerperal phlegmasia, alba dolens, embolus, sudden death.			Following childbirth, not otherwise defined.			Puerperal diseases of the breast.						Puerperal septicemia.				
		Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
1911	881,138	286	3.25	8.38	490	5.56	14.36	332	3.77	9.73	632	7.17	18.52	360	4.09	10.55	46	0.52	1.35	5	0.06	0.15	2,151	24.41	63.02	1,262	14.32	36.98	3,413	38.73
1912	872,737	329	3.77	9.47	522	5.98	15.03	378	4.33	10.88	629	7.21	18.11	362	4.15	10.42	30	0.34	0.86	7	0.08	0.20	2,257	25.86	64.99	1,216	13.93	35.01	3,473	39.79
1913	881,890	377	4.27	10.80	507	5.75	14.52	374	4.24	10.71	755	8.56	21.62	332	3.76	9.51	28	0.32	0.80	11	0.12	0.32	2,384	27.03	68.27	1,108	12.56	31.73	3,492	39.60
1914	879,096	403	4.58	10.99	465	5.29	12.68	396	4.50	10.80	659	7.50	17.97	332	3.78	9.05	40	0.46	1.09	7	0.08	0.19	2,302	26.19	62.78	1,365	15.53	37.22	3,667	41.71
1915	814,614	365	4.48	10.71	465	5.71	13.64	409	5.02	12.00	626	7.68	18.37	292	3.58	8.57	34	0.42	1.00	16	0.20	0.47	2,207	27.09	64.76	1,201	14.74	35.24	3,408	41.84
Total	4,329,475	1,760	4.07	10.08	2,449	5.66	14.03	1,889	4.36	10.82	3,301	7.62	18.91	1,678	3.88	9.61	178	0.41	1.02	46	0.11	0.26	11,301	26.10	64.75	6,152	14.21	35.25	17,453	40.31

Table II is condensed from Farr's table of the annual mortality rates for this thirty year period as given on page 249 of the Thirty-ninth Annual Report.

Farr blamed the high mortality of childbearing upon the poorly trained midwives, for in the experience of the Royal Maternity Charity, with two physicians and twenty-two trained and supervised midwives, of 9,019 deliveries of women in their homes during the three year period 1875 to 1877 there were lost only 21 women or 2.33 per 1,000 deliveries. The Birmingham Lying-in-Charity, in 8.607 deliveries, had a maternal mortality of 2.32 per 1,000. In 4.390 deliveries at Canterbury, Mr. Ridgen lost only 2.05 mothers per 1,000.

For comparison with the foregoing and with tables for the United States which follow, the subjoined table is presented, giving the mortality in childbirth per 10,000 living children, both total and under the rubrics 134–141, inclusive, of the international classification of the causes of death, for England and Wales for the five year period 1911 to 1915, as stated in the annual reports of the Registrar-General (see Table III).

The annual reports of the Registrar-General for England and Wales still carry rates of childbed mortality calculated on the basis of children reported born alive.

From the Swedish figures, notable for the care and accuracy with which they have been collected since 1746, Hendriks (1) has calculated rates showing the proportion of maternal deaths in childhood to women delivered, from 1776 to 1855, from which Table IV has been constructed.

TABLE IV.

Years.	Deaths to 100 deliveries.	Years.	Deaths to 100 deliveries.
1776–1780	0.89	1816–1820	0.73
1781–1785	0.83	1821–1825	0.67
1786–1790	0.93	1826–1830	0.66
1791–1795	1.01	1831–1835	0.57
1796–1800	0.85	1836–1840	0.51
1801–1805	0.78	1841–1845	0.44
1806–1810	0.89	1846–1850	0.44
1811–1815	0.79	1851–1855	0.44

It would appear by inference to be probable, though he does not so state, that in arriving at his figures for "women delivered," Hendriks made allowances for plural and for still births.

The writings of Mathews Duncan and of various other obstetrical writers on this subject may for the present purpose be dismissed as being based upon data of questionable accuracy, or of selected material or, of insufficient numbers. Some of the material is of value, however; as, for instance, that from certain lying-in charities and private practitioners, of which those quoted from Farr will serve as examples, for account is taken of the total number of deliveries and the maternal fatalities are presumably limited to those associated with labor.

The recent interesting article of Dublin (2), in which are compared the deaths from puerperal diseases occurring among a selected but large group of women insured in an industrial insurance company, with similar deaths among women in the registration area of the United States, has only an indirect bearing upon the questions under consideration, for the calculations are based upon the number of "females of the childbearing ages, namely, fifteen to forty-four."

However, none of the rates above considered are specific in the sense that they indicate with certainty the actual danger of childbearing, and to determine their real significance it is necessary to analyze the factors entering into both divisor and dividend. If the divisor embraced only the sum of the living and still births actually occurring, it would, when corrected for the number of plural births, represent the total number of pregnancies, which, developing to or near to the period of viability, eventuate in natural or artificially induced labor. Since the births of fetuses in the earlier months of pregnancy are not included, the total number of conceptions is not measured. Therefore, such figures are applicable not for determining the risk to life involved in becoming pregnant, but for ascertaining the risk run by those pregnant women who bear either live children or "stillborn" children. Hence, it is essential to establish just what the term *stillborn* defines.

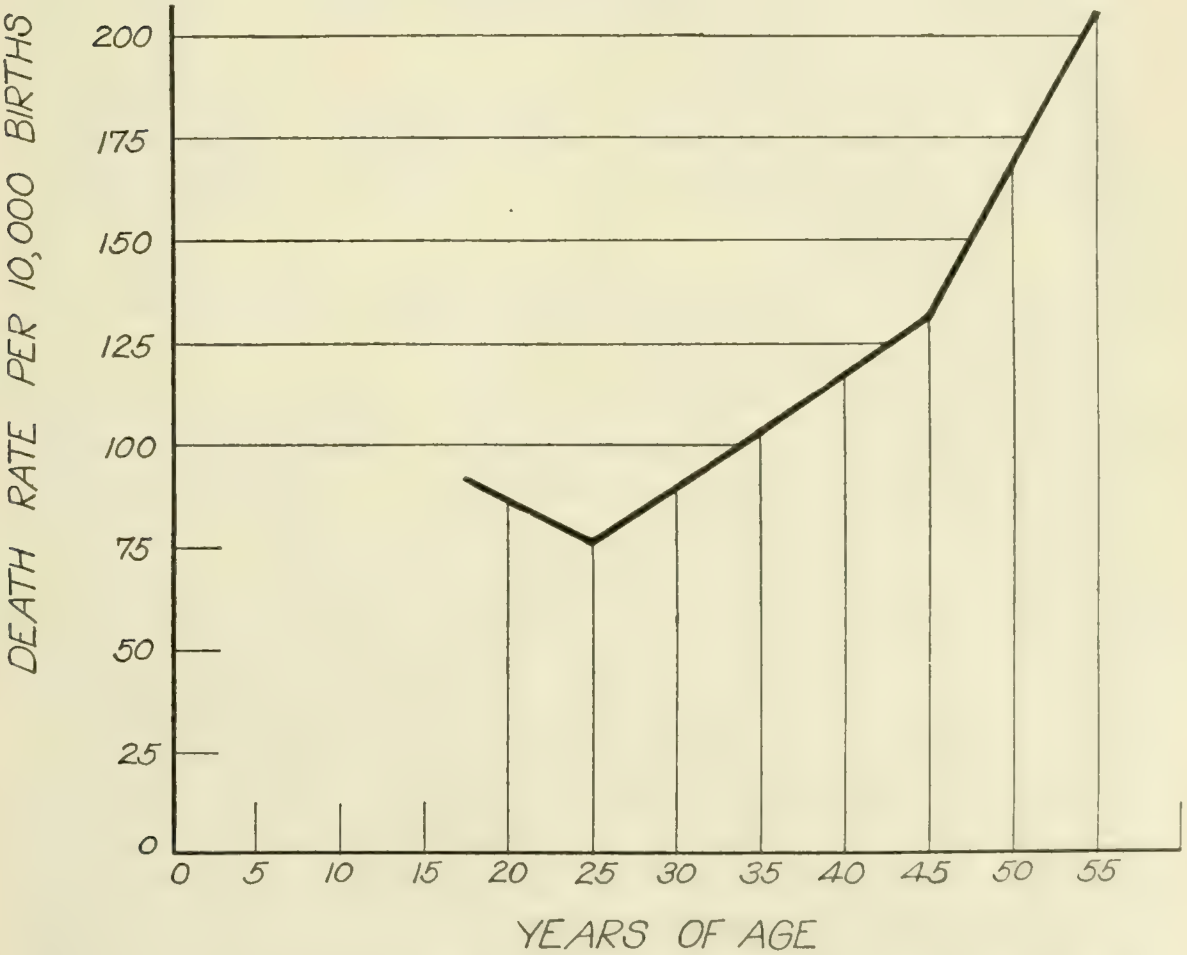
In the *Report of the Bureau of the Census on Birth Statistics* for the Birth Registration Area of the United States, for 1918, it is pointed out (pages 28 and 29) that the data relating to stillbirths must be interpreted with caution. Since the term *stillbirth* is not used in the same sense in the various states, the percentage of completeness of these reports is not known. The "model law" reads: "A stillborn child shall be registered as a birth and also as a death . . . provided that a certificate of birth and a certificate of death shall not be required for a child that has not advanced to the fifth month of uterogestation." Minnesota, New York, and North Carolina have similar provisions; the District of Columbia requires a certificate for a fetus "passed the fifth month"; the Indiana law reads "seven months gestation and over"; and the remaining states use the term *stillbirth* without defining it. Since Maryland makes especial efforts to secure uniform registration of stillbirths of even the earlier months of utero-gestation, the data for this state are not considered comparable with those of other states. It is held, however, that, since the figures of those states requiring reports only of children who have reached the fifth month approximate the figures of those states which do not define the term *stillbirth*, the conclusion is warranted that in the latter states only the stillbirths of the later months are for the most part reported; therefore, the figures are probably comparable. It is argued that the stillbirths of the later months of gestation must be well reported, because such stillbirths are interred, and burial cannot legally take place without a burial permit issued upon a physician's certificate. The data relating to stillbirths were compiled from transcripts of stillbirths reported as births, which has certainly resulted in figures smaller than would have been obtained had the transcripts of stillbirths reported as deaths been used.

Under the terms above outlined, stillbirths include some fetuses born dead before the period of viability. Since some physicians and midwives, in a small but not accurately known proportion of instances, do return as stillbirths the births of embryos of less than the fifth month of gestation, and others neglect to report the birth of some fetuses advanced beyond the fifth month and born dead, it is probably a fair assumption that these two factors balance each other, and consequently that the number of instances of stillbirths so reported represent a fair approximation of the actual number of fetuses of five months and over of utero-gestation born dead, in the birth registration area as a whole.

The number of living births reported does not, of course, in all, or perhaps in any, of the divisions of this registration area, represent the whole number of children born alive, for the reporting of live births is probably nowhere in the United States absolutely accurate. On the basis of the figures for plural and single births (both live and still) as given in the *Report of the Bureau of the Census on Birth Statistics* for 1918, the proportion of the former to the latter is as 1.15 to 100, or approximately 1 per cent.—the same percentage found by Farr for England and Wales, in 1852. It would, therefore, tend rather to decrease than to add to accuracy, to attempt to correct the figures for live births by counting the birth products of each plural pregnancy as a single unit. Attention must be called to two additional factors, the value of which is not in the present state of knowledge exactly ponderable—there must be greater risk involved in the parturient act for two or more children than for one, even should it be found that multiple pregnancy does not in itself involve greater danger to the mother than single pregnancy; and, no allowance is made for the difference in the danger between first and succeeding pregnancies. The birth rate, i.e., the rate of reported live births per 1.000 total living population, for the whole birth registration area was 24.4 in 1918, a figure, which, when compared with New York City (23.51 for 1918) where the birth registration is known to be exceptionally complete, would signify that the registration of births in the registration area is fairly accurate.

With these explanations and reservations, it may be concluded that the sum of the reported live and still births, as compiled by the Bureau of the Census, furnishes a fairly accurate, though not an absolutely correct, estimate of the total number of single labors occurring among women five months pregnant and over, in the birth registration area during 1918.

The dividend includes all the deaths classified under rubrics 134 to 141, inclusive, of the international classification of the causes of death, occurring in the cities and rural districts of the states of the birth registration area. Except where otherwise stated (in the case of certain cities in Tables VIII, IX, X, XI, XII, and XIII), the figures were obtained from the Mortality Statistics of the Bureau of the Census for 1918, which, in turn are compiled in the Bureau of the Census from transcripts of the original death certificates returned to local registrars. The classification of the deaths under the various rubrics may thus be considered as done with consistency for the whole registration area, after the certificates have been subjected to a double scrutiny to provide against error fraudulent or otherwise. Throughout this classification, as in that of the English tables, the deaths are classified specifically. Deaths of childbearing women due to other and ordinary causes are excluded; for example, deaths of childbearing women from influenza, phthisis, heart disease, cancer, etc., are classified under the appropriate primary cause—the childbearing state being reckoned a secondary cause. The influenza epidemic of 1918, therefore, had no appreciable influence upon the number of deaths classified as due to childbirth. As they stand, however, neither the total figures nor those under the separate rubrics can be accepted as representing accurately the deaths occurring in women bearing children of five months fetation and over, since these figures represent the recorded deaths of women dying in any part of the pregnant state from causes connected specifically with that condition. Under some of the eight rubrics, especially numbers 134, 136, 137, 138, and 139, there are probably recorded a not inconsiderable number of deaths of women before the fifth month of pregnancy. Accidents of pregnancy (134) includes accidental abortions, miscarriages, extra-uterine pregnancy, and vomiting of pregnancy; puerperal hemorrhage (135) may be fatal at any period of pregnancy. Some deaths due to deformities of mother or child, to injuries and operations (136), and to puerperal infection (137) undoubtedly occur before the fifth month of pregnancy, and the same may be said of the toxemias, including renal



GRAPH I illustrating death rate of mothers per 10,00 births, in reference to age.

affections and convulsions (138). Any of the various conditions enumerated under 139 may occur after abortions and miscarriages. The last two rubrics include but a small proportion of the total fatalities. Hence, the total number of deaths officially classified under the puerperal state (rubrics 134 to 139) represents not accurately, but only approximately, the number of deaths from these causes, of women five months pregnant and over.

Therefore, in both divisor and dividend, there are minor errors for which adequate allowances for correction cannot be made under the conditions by which the data are collected and recorded. It is equally clear, however, that the method here employed should measure much more accurately than any hitherto used the risk-rate to women five months pregnant and over of dying from causes connected with childbearing.

The field being cleared in the foregoing historical review and the detailed exposition of the factors involved presented, the application of the proposed method to the analysis of the data from various sources will be undertaken.

Risk-rate of childbearing in the birth registration area of the United States.

Since it furnishes a material larger in size and more homogeneous in character than any other available, the birth registration area of the United States for 1918 will be considered first. This area comprises the District of Columbia and the following states: Connecticut, Indiana, Kansas, Kentucky, Maine, Maryland, Massachusetts, Michigan, Minnesota, New Hampshire, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, Utah, Vermont, Virginia, Washington, and Wisconsin. It is estimated that this area contains 53 per cent. of the population of the United States. In it are situated besides the great cities of the Atlantic coast and of the Great Lakes region (excluding Chicago, however), the great number of smaller cities of New England, New York, Pennsylvania, Ohio, Kentucky, and of some of the western states. Classed as "cities" are those places of 10,000 inhabitants or more, according to the census of 1910. Since there were many changes in the eight years which elapsed, the distinction between urban and rural population is not so exact as would be desirable. The rural population, therefore, is by no means so truly rural—that is, village and farm dwelling—as this term is usually taken to imply. The frankly rural portion of the population dwells under very widely varying conditions of density, of climate, and of economic status, and, as is well known, it is certainly not homogeneous as to race stock. There is also, on the whole, but relatively little uniformity of the urban population in these and other important particulars. In regard to both urban and rural populations there is the greatest diversity in the character of obstetrical care. The total number of births, 1,412,283, for the area under consideration in 1918 is almost equally divided between urban and rural communities.

The figures for the maternal deaths are taken from the Mortality Statistics of the Bureau of the Census for 1918. To the possible criticism that during this year the number of maternal deaths was seriously influenced by the great epidemic of influenza, it may be said that, both in theory and in practice, this factor is eliminated in the classification of the deaths by the Bureau of the Census. The tables for New York, Philadelphia, and Baltimore to be presented later clearly indicate, so far as the official classification shows, that influenza did not affect the number of deaths from causes connected with childbirth. At any rate, choice here is limited by necessity.

The results of the analysis of the whole birth registration area are set forth in Table V. The death rates, whether viewed in comparison with the rates for England and Wales or considered alone, are appallingly high for total deaths and for deaths under each important category. These rates, although calculated by a method more favorable than that used in comparative tables in which stillbirths are not taken into account, are probably unparalleled in modern times in a civilized country. To match the rate for the whole puerperal state (88.48 per 10,000 births), it is necessary to revert to the Swedish rate for 1806–1810 (89 per 10,000 births). It is more than double the Swedish rate for 1851–1855, 75 per cent. greater than the average rate for England and Wales for 1847 to 1876 (50 per 10,000 living births), and 120 per cent. greater than the average rate for the latter area for 1911–1915 (40.31 per 10,000 living births), though the rate for the United States birth registration area—in which an allowance of 3.44 per cent. is made for stillbirths—is calculated on terms more favorable than that for England and Wales.

Considering first the rates for the United States birth registration area as a whole, it is evident that the risk to mothers in childbirth is excessive not only from all causes (88.48 per 10,000 deliveries, or 1 in 113), but from nearly every one of the separate causes. The rate for puerperal fever is 24.59 per 10,000 or 1 in every 407 deliveries, and for

TABLE V.

Maternal death rates per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by the causes under each particular rubric to the whole number of such deaths, together with the percentage of still to total births, in the birth registration area of the United States for 1918.

1918					134			135			136			138			139			140			141						137				
	Births.				Accidents of pregnancy.			Puerperal hemorrhage.			Other accidents of labor.			Puerperal albuminuria and convulsions.			Puerperal phlegmasia, alba dolens, embolus, sudden death.			Following childbirth, not otherwise defined.			Puerperal diseases of the breast.			Total puerperal state, excluding puerperal septicemia.			Puerperal septicemia.			Total puerperal state.	
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
Urban.	710,886	686,561	24,325	3.42	1,542	21.69	23.40	437	6.15	6.63	1,126	15.84	17.10	1,290	18.15	19.58	158	2.22	2.40	35	0.49	0.53	8	0.11	0.12	4,596	64.65	69.75	1,993	28.04	30.25	6,589	92.69
Rural..	701,397	677,088	24,309	3.47	1,492	21.27	25.26	417	5.95	7.06	1,133	16.15	19.18	1,206	17.19	20.42	149	2.12	2.52	27	0.38	0.46	3	0.04	0.05	4,427	63.12	74.94	1,480	21.10	25.06	5,907	84.22
Total..	1,412,283	1,363,649	48,634	3.44	3,034	21.48	24.28	854	6.05	6.83	2,259	16.00	18.08	2,496	17.67	19.97	307	2.17	2.46	62	0.44	0.50	11	0.08	0.09	9,023	63.89	72.21	3,473	24.59	27.79	12,496	88.48

all other causes combined, 63.89 per 10,000, or 1 in every 156 deliveries. Of all the women delivered, there dies 1 in 565 of puerperal albuminuria and eclampsia, 1 in 411 of accidents of pregnancy, and 1 in 625 of accidents of labor. The various prominent causes stand in proportion of their relation to the whole number of childbed deaths as follows.

	Per Cent.
Puerperal fever	27.79
Accidents of pregnancy	24.28
Puerperal albuminuria and convulsions	19.97
Accidents of labor	18.08
Puerperal hemorrhage	6.85

The other causes are of comparatively slight importance.

To return to the comparison with England and Wales, in order to form a correct idea of the facts disclosed by this analysis, the rates in the birth registration area of the United States exceed the rates in England and Wales (see Table III) by nearly 75 per cent. for puerperal fever and 150 per cent. for the other affections of the puerperal state combined. In the important categories of the latter group, accidents of pregnancy and labor, the rates for the United States birth registration area as compared with the English rates are greater by over five times for the former and nearly four times for the latter. There is but little difference between the rates in the two countries for puerperal hemorrhage. Puerperal fever is responsible for 27.79 per cent. of the whole number of deaths in the puerperal state in the United States birth registration area and for 35.24 per cent. in the English, while all other causes in the puerperal state cover 72.21 per cent. in the former and 64.75 per cent. in the latter. (See Tables III and V.)

It will be noted that for the birth registration area of the United States, the total rate and the rate for puerperal septicemia are considerably lower in the rural than in the urban communities, while the rates for the other separate categories are approximately the same; therefore, it would appear that the more favorable rural total rate is due chiefly to the marked difference in the proportion of deaths from puerperal septicemia in rural as compared with urban districts. These urban rates for the birth registration area are considerably higher in each category than the corresponding rates for the large eastern medical centres, New York, Philadelphia, Boston, and Baltimore. (See Tables X, XI, XII, and XIII.) This difference is very

TABLE VI.

Maternal death rates per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by the causes under each particular rubric to the whole number of such deaths, together with the percentage of still to total births, calculated for white and colored, urban and rural, in Kentucky, Maryland, North Carolina, and Virginia, for 1918.

	Births.				134 Accidents of pregnancy.			135 Puerperal hemorrhage.			136 Other accidents of labor.			138 Puerperal albuminuria and convulsions.			139 Puerperal phlegmasia, alba dolens, embolus, sudden death.			140 Following childbirth, not otherwise defined.			141 Puerperal diseases of the breast.			Total puerperal state, excluding puerperal. septicemia.			137 Puerperal septicemia			Total Puerperal state.	
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
Urban White	33,512	32,749	763	2.28	92	27.45	25.34	15	4.48	4.13	53	15.82	14.60	93	27.75	25.62	6	1.79	1.65	2	0.60	0.55	—	—	—	261	77.88	71.90	102	30.44	28.10	363	108.32
Rural white	154,669	149,364	5,305	3.43	338	21.85	27.61	84	5.43	6.86	244	15.78	19.93	270	17.46	22.06	11	0.71	0.90	6	0.39	0.49	2	0.13	0.16	955	61.74	78.02	269	17.39	21.98	1,224	79.14
Urban colored	9,006	8,360	646	7.17	16	17.77	9.76	6	6.66	3.66	21	23.32	12.80	54	59.96	32.93	7	7.77	4.27	—	—	—	1	1.11	0.61	105	116.59	64.02	59	65.51	35.98	164	182.10
Rural colored	47,811	44,565	3,246	6.79	100	20.92	17.76	30	6.27	5.33	107	22.38	19.04	179	37.44	31.79	6	1.25	1.07	—	—	—	—	—	—	422	88.26	74.96	141	29.49	25.04	563	117.76
Total white	188,181	182,113	6,068	3.22	430	22.85	27.10	99	5.26	6.24	297	15.78	18.71	363	19.29	22.87	17	0.90	1.07	8	0.43	0.50	2	0.11	0.13	1,216	64.62	76.62	371	19.72	23.38	1,587	84.33
Total colored	56,817	52,925	3,892	6.85	116	20.42	15.96	36	6.34	4.95	128	22.53	17.61	233	41.01	32.05	13	2.29	1.79	—	—	—	1	0.18	0.14	527	92.75	72.49	200	35.20	27.51	727	127.95
Total urban	42,518	41,109	1,409	3.31	108	25.40	20.49	21	4.94	3.98	74	17.40	14.04	147	34.57	27.89	13	3.06	2.47	2	0.47	0.38	1	0.24	0.19	366	86.08	69.45	161	37.87	30.55	527	123.95
Total rural	202,480	193,929	8,551	4.22	438	21.63	24.51	114	5.63	6.38	351	17.34	19.64	449	22.18	25.13	17	0.84	0.95	6	0.30	0.34	2	0.10	0.11	1,377	68.01	77.06	410	20.25	22.94	1,787	88.26
Total....	244,998	235,038	9,960	4.07	546	22.29	23.60	135	5.51	5.83	425	17.35	18.37	596	24.33	25.76	30	1.22	1.30	8	0.33	0.35	3	0.12	0.13	1,743	71.14	75.32	571	23.31	24.68	2,314	94.45

likely due in part to poorer obstetrical care in the large cities of the interior and in the smaller cities throughout the territory included in the registration area.

Since the figures for the still and live births and for the maternal deaths in the puerperal state are tabulated for white and colored in the registration states of Kentucky, Maryland, North Carolina, and Virginia, it is possible to determine the difference in the force of mortality in childbirth in white and colored women under both urban and rural conditions. The results of the calculations are shown in Table VI. Because there are but few Mongolian and Indian females in the populations of these states, the term *colored* may for all practical purposes be taken to express women of negro or partly negro stock. Since the chief purpose of the comparison is to investigate influences of race stock upon childbirth mortality, *negro* is used in these remarks instead of the official word *colored.* Though in the area now under consideration the urban population numbers somewhat less than one fifth of the rural, nevertheless the sample of the former is of considerable size and includes Baltimore, Richmond, and Louisville, comparatively large cities with conspicuously large negro populations. In the four states, the total rate and the urban and rural rates for all causes connected with the puerperal state are considerably in excess of those of the whole registration area. The rates for total deaths stand in order of their favorableness: white rural, total white, white urban, colored rural, total colored, colored urban. For puerperal septicemia, the total and the total rural and the rural white and the total white compare favorably with those for the registration area as a whole (23.31, 20.25, 17.39, and 19.72, respectively, to 24.59). The differences in the ratio for this cause between urban and rural rates for total, white, and colored, and particularly for the latter, is marked. The total colored is nearly double that of the total white. For the total puerperal state, puerperal fever excluded, the total rate and the urban and rural rates all are higher than those for the whole registration area. The urban rate is always higher than the rural, and the colored rate, than the white. While the accidents of pregnancy show a higher rate in the white than in the negro, the reverse is true for accidents of labor—a set of causes more particularly associated with deformities and with delivery. Puerperal hemorrhage is in none of the groups a conspicuous cause of death, the average being 5.83 per cent. of the total deaths. It is less fatal in the city dwellers than in the rural dwellers of both white and negro races, and in the white, less fatal for the former than for the latter community. Other accidents of labor, causing over 18 per cent. of the total deaths, has approximately identical rates for city and country in both races, but is greater by a third for negroes than for whites. Puerperal albuminuria and convulsions, responsible for 25.76 per cent. of the deaths and markedly higher in the urban than in the rural population for both races, is twice as fatal for the negro as for the white woman. The other two categories of causes play parts too minor to make them of serious account.

In these four states, in which the Anglo-Saxon stock greatly preponderates among those of white blood, and notwithstanding the relatively favorable influence exerted by the lower rate among the whites in Baltimore (62.02 per 10,000 deliveries), the death rate in childbearing (84.33 per 10,000), though somewhat lower than in the registration area as a whole (88.48 per 10,000), is markedly higher than any recorded for England and Wales since 1842 and nearly twice that of Sweden in 1855. The total rate for negroes (127.95 per 10,000) is considerably higher than the same rate in Baltimore (109.54 per 10,000), and the urban rate (182.10 per 10,000) is 55 per cent. greater than the rural rate (117.76 per 10,000.) The urban rate is nearly 2 per 100, or about 1 in 45 deliveries, while in the country 1 out of about every 58 childbearings is fatal to the mother. Since the proportion of stillborn is so high among the negroes, the sacrifice of mothers in producing live children is even greater than these figures indicate. Though further and more intensive studies based upon accurate and complete histories of a sufficient number of cases are necessary to determine definitely the reasons for the greatly higher maternal mortality in the negro over the white in childbearing, the evidence at hand suggests that the causes at work are concerned in considerable degree, at least, with difference in degree of racial susceptibility to puerperal infection, albuminuria and convulsions (the toxemias of pregnancy and eclampsia), and to accidents of labor. The rates of Table VI show that, whether in city or in country, the mortality from these causes is much in excess among the negroes.

Influence of age upon risk-rate of childbearing in the birth registration area of the United States.

In Table VII, the births and the maternal deaths by age periods and the rates calculable therefrom are given for the birth registration

TABLE VII.

Maternal death rates per 10,000 births (live and still) according to age of mother, from causes connected with the puerperal state, and the percentage contributed by the causes under each particular rubric to the whole number of such deaths, together with the percentage of still to total births, for the birth registration area of the United States, exclusive of Maine, Massachusetts, Rhode Island, and Washington, for 1918.

(Data on stillbirths for Baltimore, Maryland, obtained from the Baltimore Health Department.)

Age period.	Births.				134 Accidents of pregnancy.			135 Puerperal hemorrhage.			136 Other accidents of labor.			138 Puerperal albuminuria and convulsions.			139 Puerperal phlegmasia, alba dolens, embolus, sudden death.			140 Following childbirth, not otherwise defined.			141 Puerperal diseases of the breast.			Total puerperal state, excluding Puerperal septicemia.			137 Puerperal septicemia.			Total puerperal state.	
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
10–19....	102,768	98,232	4.536	4.41	183	17.81	19.26	22	2.14	2.32	132	12.84	13.89	317	30.85	33.37	8	0.78	0.84	3	0.29	0.32	1	0.10	0.11	666	64.81	70.11	284	27.64	29.89	950	92.44
20–29....	705,563	681,926	23,637	3.35	1,488	21.09	27.68	244	3.46	4.54	1,009	14.30	18.77	1,007	14.27	18.73	112	1.59	2.08	28	0.40	0.52	6	0.09	0.11	3,894	55.19	72.43	1,482	21.00	27.57	5,376	76.19
30–39 ...	379,295	363,575	15,720	4.14	899	23.70	23.00	373	9.83	9.54	684	18.03	17.50	704	18.56	18.01	103	2.72	2.64	17	0.45	0.44	2	0.05	0.05	2,782	73.35	71.19	1,126	29.69	28.81	3,908	103.03
40–49....	55,807	52,468	3,339	5.98	99	17.74	13.52	116	20.79	15.85	120	21.50	16.39	167	29.92	22.81	23	4.12	3.14	—	—	—	1	0.18	0.14	526	94.25	71.86	206	36.91	28.14	732	131.17
50 and over...	243	219	24	9.88	1	41.15	20.00	—	—	—	1	41.15	20.00	1	41.15	20.00	—	—	—	—	—	—	—	—	—	3	123.46	60.00	2	82.30	40.00	5	205.76
Unknown	9,974	5,448	1,927	19.32	5	5.01	25.00	3	3.01	15.00	3	3.01	15.00	4	4.01	20.00	—	—	—	—	—	—	—	—	—	15	15.04	75.00	5	5.01	25.00	20	20.05
Total .	1,251,723	1,201,868	49,855	3.98	2,675	21.37	24.34	758	6.06	6.90	1,949	15.57	17.73	2,200	17.58	20.02	246	1.97	2.24	48	0.38	0.44	10	0.08	0.09	7,886	63.00	71.75	3,105	24.81	28.25	10,991	87.81

area as of 1918, with the omission of certain states—Maine, Massachusetts, Rhode Island, and Washington, for which comparative data could not be obtained. On account of the manner of age classification of deaths in the Mortality Reports of the Bureau of the Census, it is not possible to begin in the ten year periods with the fifteenth year as in Farr's Table.

It will be noted that for the first age group, 10 to 19 years, in which about one twelfth of the births occur, the total rate is higher than for the second ten year period, 20 to 29 years, but lower than for any other of the age divisions. The maternal death rates in this group, in which most of the labors probably occur in primiparae, are, in comparison with the second and third age groups, relatively low for all the important categories embraced under "total puerperal state, excluding puerperal fever," with the exception of the one category—albuminuria and convulsions. The rate for puerperal fever and the total rate are higher, and the rates for accidents of pregnancy and labor and for hemorrhage are lower than for the averages of all ages.

The second age group, in which over half of the labors fall, shows the lowest rates for total and for the important rubrics of septicemia and albuminuria and convulsions, and, in contrast to the succeeding age groups, in the rate for accidents of labor; for this category, as with all the other important ones, it is below that of the average for all ages. So far as can be determined by this method of age classification, this is, on the whole, the most favorbale age for childbearing.

The third and fourth age groups, 30 to 39 years and 40 to 49 years, including over a third of the labors, show in most of the categories progressively higher rates—the increase of the risk of death from hemorrhage, albuminuria and convulsions, and puerperal fever being conspicuous at these higher ages. The total rates are in consequence higher than at other ages.

The age group "over 50 years," with high rates in all the important categories, particularly in septicemia, has a total rate of 206.68 per 10,000. The sample, however, is too small to warrant fast conclusions.

The relative importance in the various age groups of the different categories is shown in the columns of percentages. The value of these from the practical standpoint of prognosis would be considerably enhanced, if it were possible to make the age groupings by quinquennial periods. The influence of the age of the mother upon the death rates from all causes connected with the puerperal state is indicated in Graph I (p. 207).

THE RISK-RATE OF CHILDBEARING IN CERTAIN CITIES OF EUROPE AND THE UNITED STATES.

In the annual reports of European cities, which so far I have been able to obtain, there are only two, Stockholm, Sweden, and Birmingham, England, in which the stillbirths are reported and the maternal deaths are given under the separate rubrics over a series of years. For comparison with similar data for the below mentioned cities of the United States, I have compiled tables for these cities, showing the total rates and the rates for the different categories under deaths in the puerperal state. The data for these cities and for New York, Philadelphia, Boston, and Baltimore were obtained from the annual official health reports. Therefore, since the deaths were not classified in the Bureau of the Census, there may be some difference in their assignment in some of the categories, due to variations in custom or of opinion in the several statistical bureaus.

It will be observed that the classification used in Stockholm is somewhat different from that of the international classification and with fewer rubrics. Apparently, however, the latter include all the deaths occurring in the puerperal state (see Table VIII). Had the table for Stockholm been made to include the years 1910 to 1914, the showing for the city would have been even more favorable. Indeed, when the steady upward progress of the rates for puerperal septicemia since 1914, causing a doubling of the rate for that category and a large increase in the total rate, is taken into consideration, it would appear that during the World War factors favorable for this affection have been active in Stockholm, temporarily affecting the rates to a disadvantage. Hemorrhage, except from placenta previa, is probably included under "other accidents of labor." The rates for the total puerperal state are remarkably low. The unusually excellent results in the management of labor in Stockholm are probably explained partly by the physique of the women and partly by good obstetrical practice and the large extent to which hospitalization is exercised. The low percentage of stillbirths to total births supports this view.

The death rates for the puerperal state for Birmingham are probably among the lowest reached by any large city and are the result of efforts intelligently directed over a long series of years (see

TABLE VIII.

Maternal death rates per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by causes under each particular rubric to the whole number of such deaths, together with the percentage of stillbirths to total births, for Stockholm, Sweden, 1915–1919

(Compiled from the annual reports of the Health Department of Stockholm.)

Year.	Births.				Abortion, difficult labor.			Placenta previa.			Convulsions, eclampsia.			All other accidents of labor.			Total puerperal state, excluding puerperal septicemia.			Puerperal septicemia.			Total Puerperal state.	
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
1915...	7,082	6,913	169	2.39	4	5.65	20.00	–	—	—	1	1.41	5.00	2	2.82	10.00	7	9.88	35.00	13	18.36	65.00	20	28.24
1916...	6,736	6,579	157	2.33	2	2.97	8.33	–	—	—	3	4.45	12.50	4	5.94	16.67	9	13.36	37.50	15	22.27	62.50	24	35.63
1917...	6,758	6,634	124	1.83	–	—	—	–	—	—	2	2.96	9.52	1	1.48	4.76	3	4.44	14.29	18	26.64	85.71	21	31.07
1918...	6,026	5,887	139	2.31	2	3.32	5.71	2	3.32	5.71	3	4.98	8.57	2	3.32	5.71	9	14.94	25.71	26	43.15	74.29	35	58.08
1919...	5,560	5,454	106	1.91	–	—	—	–	—	—	2	3.60	6.67	3	5.40	10.00	5	8.99	16.67	25	44.96	83.33	30	53.96
Total..	32,162	31,467	695	2.16	8	2.49	6.15	2	0.62	1.54	11	3.42	8.46	12	3.73	9.23	33	10.26	25.38	97	30.16	74.62	130	40.42

TABLE IX.

Maternal death rates per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by the causes under each particular rubric to the whole number of such deaths, together with the percentage of stillbirths to total births, for Birmingham, England, for 1913, 1914, 1915, 1916, and 1918.

(Compiled from the annual reports of the Health Department of Birmingham.)

Years.	Births.				134			135			136			138			139			140			141						137				
					Accidents of pregnancy.			Puerperal hemorrhage.			Other accidents of labor.			Puerperal albuminuria and convulsions.			Puerperal phlegmasia, alba dolens, embolus, sudden death.			Following childbirth, not otherwise defined.			Puerperal diseases of the breast.			Total puerperal state, excluding puerperal septicemia.			Puerperal septicemia.			Total puerperal state.	
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
1913...	24,491	23,812	679	2.77	8	3.27	8.69	14	5.72	15.22	10	4.08	10.87	9	3.67	9.78	7	2.86	7.61	—	—	—	—	—	—	48	19.60	52.17	44	17.97	47.83	92	37.56
1914 ..	22,600	21,838	762	3.37	9	3.98	12.16	10	4.42	13.51	11	4.87	14.86	9	3.98	12.16	2	0.88	2.70	—	—	—	—	—	—	41	18.14	55.41	33	14.60	44.59	74	32.74
1915 ..	21,919	21,187	732	3.34	6	2.74	8.22	10	4.56	13.70	8	3.65	10.96	10	4.56	13.70	3	1.37	4.11	—	—	—	1	0.46	1.37	38	17.34	52.05	35	15.97	47.95	73	33.30
1916 ..	21,347	20,618	729	3.41	2	0.94	2.82	10	4.68	14.08	5	2.34	7.04	13	6.09	18.31	9	4.22	12.68	1	0.47	1.41	—	—	—	40	18.74	56.34	31	14.52	43.66	71	33.26
1918..	17,430	16,840	590	3.38	4	2.29	7.84	8	4.59	15.69	3	1.72	5.88	4	2.29	7.84	3	1.72	5.88	—	—	—	—	—	—	22	12.62	43.14	29	16.64	56.86	51	29.26
Total.	107,787	104,295	3,492	3.24	29	2.69	8.03	52	4.82	14.40	37	3.43	10.25	45	4.17	12.47	24	2.23	6.65	1	0.09	0.28	1	0.09	0.28	189	17.53	52.35	172	15.96	47.65	361	33.49

Table IX.) It will be noted that the total rate has consistently fallen through the five-year period, three and one half years of which were during the World War. The rate for each of the important categories is strikingly low. It is evident, when comparison is made with the rates of New York, Philadelphia, Boston, and Baltimore, and with those of the birth registration area of the United States, that the low rates of this manufacturing city must, in large degree, be due to the character of obstetrical care afforded. These rates are not only so low, but relatively so well balanced, that they will be taken as standards of comparison for the American cities now to be discussed.

The average total rate for New York City (46.11 per 10,000 labors), conspicuously lower than that for any of the other cities of the United States under consideration, is over 35 per cent. higher than that for Birmingham (see Table X). The average rate for puerperal septicemia is not only lower than that for Birmingham, but the rate for this affection has fallen continuously throughout the five year period, and in 1919 reached the remarkably low figure of 11.41 per 10,000 labors. The reports of the Department of Health indicate that this result has been materially influenced by persistent and intelligently directed administrative measures. While the rates for the other categories are not so low as those for Birmingham, they are well below those for the other American cities in this group.

In Philadelphia, both the total rate (61.48 per 10,000 labors) and the rates for the important categories are well above those for New York—notably so for puerperal fever, the average rate (27.10 per 10,000 labors) being double that (14.41 per 10,000 labors) for the latter city (see Table XI). In accidents of labor, however, the record of Philadelphia is lower by half, but this is evidently due to classifying deaths under 140 which in New York would be assigned to 136.

The rates for Boston, for 1914 to 1918, are given in Table XII. The average rate for all causes connected with the puerperal state (70.71 per 10,000, or 1 in 141 births) is excessively high as compared with the other cities. The average rates for all the important rubrics are considerably higher than in New York, and with the exception of puerperal septicemia they are greater than those in Philadelphia. In regard to puerperal septicemia, Boston surpasses Baltimore, in spite of the large negro population of the latter city.

The average total rate for the puerperal state in Baltimore is not only very high (64.89 per 10,000, or about 1 in every 150 deliveries),

TABLE X.

Maternal death rates per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by causes under each particular rubric to the whole number of such deaths, together with the percentage of stillbirths to total births, for New York City, 1915–1919.

(*Compiled from the annual reports of the Health Department of New York.*)

Year.	Births.				134			135			136			138			139			140			141						137				
					Accidents of pregnancy.			Puerperal hemorrhage.			Other accidents of labor.			Puerperal albuminuria and Convulsions.			Puerperal phlegmasia, alba dolens, embolus, sudden death.			Following childbirth, not otherwise defined.			Puerperal diseases of the breast.			Total puerperal state, excluding puerperal septicemia.			Puerperal septicemia.			Total puerperal state.	
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
1915	147,669	141,256	6,413	4.34	118	7.99	16.62	63	4.27	8.87	91	6.16	12.82	144	9.75	20.28	38	2.57	5.35	3	0.20	0.42	4	0.27	0.56	461	31.22	64.93	249	16.86	35.07	710	48.08
1916	143,917	137,664	6,253	4.34	78	5.42	11.94	85	5.91	13.02	69	4.79	10.57	158	10.98	24.20	40	2.78	6.13	1	0.07	0.15	1	0.07	0.15	432	30.02	66.16	221	15.36	33.84	653	45.37
1917	147,681	141,564	6,117	4.14	77	5.21	11.83	80	5.42	12.29	74	5.01	11.37	169	11.44	25.96	30	2.03	4.61	2	0.14	0.31	6	0.41	0.92	438	29.66	67.28	213	14.42	32.72	651	44.08
1918	144,839	138,046	6,793	4.69	95	6.56	14.31	83	5.73	12.50	61	4.21	9.19	184	12.70	27.71	24	1.66	3.61	10	0.69	1.51	5	0.35	0.75	462	31.90	69.58	202	13.95	30.42	664	45.84
1919	136,361	130,377	5,984	4.39	128	9.39	19.88	61	4.47	9.47	84	6.16	13.04	171	12.54	26.55	43	3.15	6.68	2	0.15	0.31	2	0.15	0.31	491	36.01	76.24	153	11.22	23.76	644	47.23
Total	720,467	688,907	31,560	4.38	496	6.88	14.93	372	5.16	11.20	379	5.26	11.41	826	11.46	24.86	175	2.43	5.27	18	0.25	0.54	18	0.25	0.54	2,284	31.70	68.75	1,038	14.41	31.25	3,322	46.11

TABLE XI.

Maternal death rates per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by causes under each particular rubric to the whole number of such deaths, together with the percentage of stillbirths to total births, for Philadelphia, 1915–1919.

(Compiled from the annual reports of the Health Department of Philadelphia.)

Year.	Births.				134 Accidents of pregnancy.			135 Puerperal hemorrhage.			136 Other accidents of labor.			138 Puerperal albuminuria and convulsions.			139 Puerperal phlegmasia, alba dolens, embolus, sudden death.			140 Following childbirth, not otherwise defined.			141 Puerperal diseases of the breast.			Total puerperal state, excluding puerperal septicemia.			137 Puerperal septicemia.			Total puerperal state.	
	Total.	Living.	Still.	Per cent of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
1915...	42,820	40,849	1,971	4.60	20	4.67	7.09	26	6.07	9.22	10	2.34	3.55	79	18.45	28.01	12	2.80	4.26	12	2.80	4.26	—	—	—	159	37.13	56.38	123	28.73	43.62	282	65.86
1916...	42,563	41,220	1,343	3.16	24	5.64	8.30	32	7.52	11.07	15	3.52	5.19	54	12.69	18.69	12	2.82	4.15	18	4.23	6.23	—	—	—	155	36.42	53.63	134	31.48	46.37	289	67.90
1917 ...	44,897	42,917	1,980	4.41	13	2.90	5.02	23	5.12	8.88	8	1.78	3.09	68	15.15	26.25	9	2.00	3.47	17	3.79	6.56	—	—	—	138	30.74	53.28	121	26.95	46.72	259	57.69
1918 ...	45,153	42,904	2,249	4.98	15	3.32	5.88	23	5.09	9.02	12	2.66	4.71	60	13.29	23.53	4	0.89	1.57	27	5.98	10.59	1	0.22	0.39	142	31.45	55.69	113	25.03	44.31	255	56.47
1919 ...	44,149	42,047	2,102	4.76	19	4.30	7.17	26	5.89	9.81	13	2.94	4.91	69	15.63	26.04	11	2.49	4.15	23	5.21	8.68	—	—	—	161	36.47	60.75	104	23.56	39.25	265	60.02
Total	219,582	209,937	9,645	4.39	91	4.14	6.74	130	5.92	9.63	58	2.64	4.30	330	15.03	24.44	48	2.19	3.56	97	4.42	7.19	1	0.05	0.07	755	34.38	55.93	595	27.10	44.07	1,350	61.48

TABLE XII.

Materna' death rates per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by causes under each particular rubric to the whole number of such deaths, together with the percentage of stillbirths to total births, for Boston, 1914 to 1918.

(Compiled from the annual report of the Health Department of Boston.)

Year.	Births.				134			135			136			138			139			140			141						137				
					Accidents of pregnancy.			Puerperal hemorrhage.			Other accidents of labor.			Puerperal albuminuria and convulsions.			Puerperal phlegmasia, alba dolens, embolus, sudden death.			Following] childbirth, not otherwise defined.			Puerperal diseases of the breast.			Total puerperal state, excluding puerperal septicemia.			Puerperal septicemia.			Total puerperal state.	
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
1914..	20,251	19,462	789	3.90	8	3.95	6.30	18	8.89	14.17	21	10.37	16.54	34	16.79	26.77	1	0.49	0.79	1	0.49	0.79	—	—	—	83	40.99	65.35	44	21.73	34.65	127	62.71
1915.	20,416	19,725	691	3.38	10	4.90	6.49	17	8.33	11.04	18	8.82	11.69	51	24.98	33.12	9	4.41	5.84	—	—	—	1	0.49	0.65	106	51.92	68.83	48	23.51	31.17	154	75.43
1916..	20,308	19,594	714	3.52	11	5.42	8.46	12	5.91	9.23	13	6.40	10.00	25	12.31	19.23	10	4.92	7.69	2	0.98	1.54	—	—	—	73	35.95	56.15	57	28.07	43.85	130	64.01
1917..	20,523	19,856	667	3.25	26	12.67	14.94	11	5.36	6.32	24	11.69	13.79	41	19.98	23.56	12	5.85	6.90	2	0.97	1.15	1	0.49	0.57	117	57.01	67.24	57	27.77	32.76	174	84.78
1918	20,890	20,063	827	3.96	21	10.05	15.11	13	6.22	9.35	23	11.01	16.55	33	15.80	23.74	8	3.83	5.76	1	0.48	0.72	—	—	—	99	47.39	71.22	40	19.15	28.78	139	66.54
Total	102,388	98,700	3,688	3.60	76	7.42	10.50	71	6.93	9.81	99	9.68	13.67	184	17.97	25.41	40	3.91	5.52	6	0.59	0.83	2	0.20	0.28	478	46.69	66.02	246	24.03	33.98	724	70.71

but unlike those of New York and Philadelphia shows an almost unbroken ascent and in 1919 is almost 40 per cent. higher than in 1915. This increase is in nearly every rubric, including septicemia. The average rate for the latter affection, however, is conspicuously lower than that for Philadelphia and for the birth registration area, but much higher than that for New York (see Table XIII). On the whole, the record for Baltimore, like that for Boston, is without a redeeming feature, even the rate for puerperal hemorrhage being about 40 per cent. above the average for that category in the other tables. On account of the large size of the negro population, Baltimore offers a good opportunity to compare the childbed death rates among negroes and whites in a large city. Negroes comprise about 2 per cent. of the populations in New York and Boston, and about 5 per cent. in Philadelphia. In Baltimore, about 15 per cent. or one sixth of both population and births, is negro. The two years, 1916 and 1919, for which data for comparison are obtainable, are by chance the years showing the lowest and the highest rates. It will be noted that the average of the total negro rate for these two years is almost double that of the white (109.54 to 62.02), the cause of the excess being the higher negro rates for accidents of labor, puerperal convulsions and albuminuria, and septicemia, all of which are extraordinarily high and double those of the whites (1 in 90 as against 1 in 160 deliveries). The rate for puerperal septicemia (40.44 per 10,000 births) in the negroes in Baltimore is higher than the total rate for the whole puerperal state in Birmingham and Stockholm, and not far from that in New York. It is interesting that the comparatively high rates for accidents of pregnancy and for puerperal hemorrhage in Baltimore are approximately the same for the two races.

According to information furnished me in 1917, about one half of the lying-in women receiving obstetrical care in the well organized in- and out-patient departments of the obstetrical clinics of the Johns Hopkins Hospital and of the University of Maryland Hospital are negroes, representing about 50 per cent. of the annual number of negro births in the city. The other half of the deliveries in these obstetrical services includes less than 10 per cent. of the white births. The deliveries in these institutions represent approximately 15 per cent. of the annual births in the City, so about 50 per cent. of the negro and about 10 per cent. of the white labors occur under these auspices. Notwithstanding these relatively very favorable opportuni-

TABLE XIII.

Maternal death rate per 10,000 births (live and still) from causes connected with the puerperal state, and the percentage contributed by causes under each particular rubric to the whole number of such deaths, together with the percentage of stillbirths to total births, for Baltimore. Corresponding rates and percentages are calculated for white and colored for 1916 and 1919.

(Compiled from the annual reports of the Health Department of Baltimore.)

Year.	Births.				134			135			136			138			139			140			141			Total puerperal state, excluding puerperal septicemia.			137			Total puerperal state.	
					Accidents of pregnancy.			Puerperal hemorrhage.			Other accidents of labor.			Puerperal albuminuria and convulsions.			Puerperal phlegmasia, alba dolens, embolus, sudden death.			Following childbirth, not otherwise defined.			Puerperal diseases of the breast.						Puerperal septicemia.				
	Total.	Living.	Still.	Per cent. of still to total.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.	Per cent. of total puerperal state.	Deaths.	Rate per 10,000 births.
1915.........	14,765	13,634	1,131	7.66	11	7.45	13.75	10	6.77	12.50	6	4.06	7.50	22	14.90	27.50	6	4.06	7.50	—	—	—	—	—	—	55	37.25	68.75	25	16.93	31.25	80	54.18
1916.........	16,320	15,085	1,235	7.57	17	10.42	16.35	12	7.35	11.54	8	4.90	7.69	28	17.16	26.92	4	2.45	3.85	—	—	—	—	—	—	69	42.28	66.35	35	21.45	33.65	104	63.73
1917.........	16,217	14,950	1,267	7.81	8	4.93	8.79	11	6.78	12.09	7	4.32	7.69	31	19.12	34.07	2	1.23	2.20	—	—	—	—	—	—	59	36.38	64.84	32	19.73	35.16	91	56.11
1918.........	16,492	15,292	1,200	7.28	30	18.19	25.21	12	7.28	10.08	14	8.49	11.76	31	18.80	26.05	1	0.61	0.84	—	—	—	—	—	—	88	53.36	73.95	31	18.80	26.05	119	72.16
1919.........	18,958	17,631	1,327	7.00	21	11.08	14.69	19	10.02	13.29	21	11.08	14.69	38	20.04	26.57	4	2.11	2.80	—	—	—	—	—	—	103	54.33	72.03	40	21.10	27.97	143	75.43
Total.........	82,752	76,592	6,160	7.44	87	10.51	16.20	64	7.73	11.92	56	6.77	10.43	150	18.13	27.93	17	2.05	3.17	—	—	—	—	—	—	374	45.20	69.65	163	19.70	30.35	537	64.89
1916 { white .	13,503	12,662	841	6.23	14	10.37	18.92	11	8.15	14.86	5	3.70	6.76	18	13.33	24.32	3	2.22	4.05	—	—	—	—	—	—	51	37.77	68.92	23	17.03	31.08	74	54.80
{ colored	2,817	2,423	394	3.99	3	10.65	10.00	1	3.55	3.33	3	10.65	10.00	10	35.50	33.33	1	3.55	3.33	—	—	—	—	—	—	18	63.90	60.00	12	42.60	40.00	30	106.50
1919 { white .	15,841	14,908	933	5.89	18	11.36	16.67	15	9.47	13.89	16	10.10	14.81	27	17.04	25.00	4	2.53	3.70	—	—	—	—	—	—	80	50.50	74.07	28	17.68	25.93	108	68.18
{ colored	3,117	2,723	394	12.64	3	9.62	8.57	4	12.83	11.43	5	16.04	14.29	11	35.29	31.43	—	—	—	—	—	—	—	—	—	23	73.79	65.71	12	38.50	34.29	35	112.29
Total white. .	29,344	27,570	1,774	6.05	32	10.91	17.58	26	8.86	14.29	21	7.16	11.54	45	15.34	24.73	7	2.39	3.85	—	—	—	—	—	—	131	44.64	71.98	51	17.38	28.02	182	62.02
Total colored	5,934	5,146	788	13.28	6	10.11	9.23	5	8.43	7.69	8	13.48	12.31	21	35.39	32.31	1	1.69	1.54	—	—	—	—	—	—	41	69.09	63.08	24	40.44	36.92	65	109.54

* *In number of stillborn white are included, 3 color unknown and 2 sex and color unknown.*

ties for lying-in care, the Baltimore negress is a very poor maternity risk. Her chances are materially better, however, than in the four states analyzed in Table VI.

In this connection, comparison may be made with Washington, D. C., rates for which I have calculated for 1918 on the basis of figures from the census reports. With 8,673 births (living and still), 6,298 white and 2,375 colored, and 74 maternal deaths, 47 white and 27 colored, the total maternal death rate was 85.32 per 10,000 births —74.63 for white and 113.68 for colored. Thus, in the National Capital, the risk rate for childbirth for the negro exceeded that in Baltimore.

STILLBIRTHS.

The question of the relative frequency of stillbirths as compared with live births is one of great interest and importance. That the proportion varies in the same country at different times and places and in different countries is to be expected. Owing to uncertainties as to what the term *stillbirth* is used to comprehend at different times and in various localities, absolute comparisons are unwarranted and minute corrections are impossible.

For the sake of comparison, figures for Sweden and other continental countries taken from the tables of Hendriks (loc. cit., p. 167) and figures from my tables V to XIII are given in Table XIV. In regard to Sweden, Hendriks showed that from 1776 to 1855, on an average to every 100 deliveries there were born 101.62 live and still born children, of which 98.80 were live and 2.80 still born; during the five year period, 1851 to 1855, these proportions were in the rural districts 101.39, 98.27, 3.12, and in towns, 101.46, 96.98, and 4.48.

It will be noted that the percentage of stillbirths for Sweden was much lower than in any of the other countries given in Hendriks table and that the percentage in Stockholm is at the present time considerably below that of any of the other cities in my table. In comparison with the experience of Swedish towns in 1851 to 1855, the stillbirth percentage of this city is now lower by one half. This relatively low percentage of stillbirths in Sweden and its chief city is probably influenced by the same factors already suggested as explaining the low death rates in maternity; i.e., the physique of the women and the superior obstetrical care. However, that low maternal death rates and low percentages of stillbirths do not of necessity go together is evident from the figures of Birmingham, New York, and Boston. The former city with its remarkably low maternity death rates has a stillbirth rate nearly as high as that of Boston, where the maternity death rates are notably high. Philadelphia, with a maternity death rate greater by more than 30 per cent. than that of New York, has the same stillbirth rate. In the birth registration area as a whole and in the four states, Kentucky, Maryland, North Carolina, and Virginia, taken together, with maternity death rates much higher than that for New York, the stillbirth rates, whether total, urban or rural are considerably lower than in that city. The stillbirth rate for the whole birth registration area of the United States is but very little higher than that of Birmingham, whose maternal death rate is, on the other hand, less by considerably more than 60 per cent.* The very high stillbirth rate of Baltimore,

TABLE IV.

Hendriks.			Howard.		
City or country.	Date.	Average percentage of still to total births.	City or country	Date.	Average percentage of still to total births.
Sweden........	1776–1855	2.77	Stockholm............	1915–1919	2.16
" Rural..	1851–1855	3.12	Birmingham...........	1913–1918	3.24
" Urban .	1851–1855	4.48	New York.............	1915–1919	4.38
Norway.......	1836–1845	4.08	Philadelphia..........	1915–1919	4.39
Denmark......	1845–1849	4.26	Boston...............	1917–1918	3.60
Hannover.....	1853	3.87	Baltimore.............	1915–1919	7.44
Prussia........	1825–1849	3.69	" White.......	"	6.05
Saxony........	1847–1849	4.49	" Negro.......	"	13.28
Bavaria.......	1844–1851	3.3	U. S. Birth Registration Area................	1918	3.44
Netherlands ...	1848–1853	4.97	U. S. Birth Registration Area, Urban.........	"	3.42
Belgium.......	1841–1850	4.18	U. S. Birth Registration Area, Rural..........	"	3.47
France........	1851–1854	3.79	Kentucky, Maryland, North Carolina, and Virginia.............	"	4.07
			Total Urban.......	"	3.31
			Total Rural........	"	4.22
			Total White.......	"	3.22
			Urban White.......	"	2.28
			Rural White.......	"	3.43
			Total Negro.......	"	6.85
			Urban Negro......	"	7.17
			Rural Negro.......	"	6.79

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* The explanation of this seeming paradox must be sought for in the operation of some potent cause of maternal death, which is at the same time without

in both white and negro is due to some degree to the custom of reporting as stillbirths fetuses of earlier months of utero-gestation, partly under the influence of the state authorities and partly because of the response of physicians to requests for stillborn fetuses for scientific investigation in the laboratories of embryology. It is probably not far from the truth to assume that on the same basis of birth reporting in other places, the percentage of still to total births in Baltimore is not far from 5 in whites and 11 in negroes—rates of fetal fatality of considerable eminence. The percentage of stillbirths to total births in Washington, D. C., for 1918, was 5.89 for the whole number, 4.40 for white, and 9.85 for negro. It is significant that here as in the four states, Kentucky, Maryland, North Carolina, and Virginia, the stillbirth rate for negroes is double that for whites. In this division of the registration area, the stillbirth rate for whites is lower in the cities than in the rural districts, while for negroes, the opposite is the case.

It has long been known that fetal fatality is influenced conspicuously by causes inherent in the fetus itself (namely, errors of implantation and of development) and by accidents *in utero*—malposition of fetus and of cord, as well as by infectious diseases of the mother or of the fetus, or of both (such as, smallpox, pneumonia, typhoid and typhus fevers, influenza, and syphilis), by deformities and various toxemias and chronic diseases of the mother, and by injuries and accidents in pregnancy and labor.

From the material now under consideration, it is not possible, of course, to determine the relative importance of these various causes of stillbirth. It is well established that the effects of many of these causes may be modified or even completely controlled by appropriate care during pregnancy and labor. Hence, to a considerable degree, the proportion of stillbirths to live births in a community may be used as an indicator of the character of obstetrical care obtained by pregnant and lying-in women. When both the maternal and fetal death rates are high, unless the race stock is particularly weak, poor obstetrical care may be assumed. When the maternal death rate is comparatively low and the stillbirth rate is high, and vice versa, it may be predicated that obstetrical care is good but inadequately balanced. When the death rates for both mother and fetus are low, efficient and well rounded obstetrical care is indicated.

influence upon infant mortality. This is found in puerperal septicemia, the one prominent cause of maternal deaths from causes connected with the puerperal state that does not ordinarily act or begin to act until some days after labor.

Causes of high maternal death rates and high stillbirth rates in the United States, and their correction.

Abundant experience teaches that high maternal and infantile death rates from causes connected with childbearing are associated with inadequate care afforded by ignorant and careless accouchers and nurses and that, conversely, low death rates obtain in the practice of well-trained and careful attendants, even under environmental conditions ordinarily considered adverse. It is known that physicians and midwives of certain levels of intelligence and education can be trained to become accomplished accoucheurs. It is equally well known that in the United States, until very recent years, diploma mills ground out some intelligent and numbers of unintelligent and poorly trained men and women physicians with hopelessly inadequate obstetrical training and that many of these are still in the full bloom of practice. It is no secret that there are in this country no properly organized schools for training midwives and that even where midwife practice is supposedly regulated by legal statute, registration, examination, and supervision are tragically farcical. Of all the important branches of the nursing art, that of obstetrics is perhaps at once the most neglected and the most important. It cannot be questioned that the risks to both mother and child, even in the care of the most intelligent and well-trained accoucheurs, are enhanced by those constitutional weaknesses, racial or individual, inherited or acquired, general or local, that influence deformities, accidents of pregnancy and labor, toxemias, and resistance to infection. There is considerable evidence that the negro race in the United States is in these respects constitutionally weaker and therefore a poorer childbirth risk than the white. To what degree this handicap is increased by poorer obstetrical care is, however, unknown, while, in the indefinite congeries of causes grouped under accidents of pregnancy in Tables VI and XIII, the rates for whites are actually higher than for negroes; in accidents of labor, puerperal albuminuria and eclampsia, and septicemia, the rates for negroes are invariably higher, and for Baltimore, there is some evidence that these differences are to a certain extent independent of the character of obstetrical care. These data are, however, inadequate for far reaching conclusions.

Why should maternal mortality rates be so much lower in the rural than in the urban communities of the United States Birth registration area and in Kentucky, Maryland, North Carolina, and Virgina, and yet lower in the large cities of New York, Philadelphia, Boston, and Baltimore than in the rural districts, in only one of which cities, New York, is there evidence that administrative measures have influenced that result—and there only in the one category, puerperal fever? To what degree is the difference between rural and urban maternity death rates due to the carrying of the worst risks to "cities" for obstetrical care? What is the difference between the relative distribution of midwife and medical obstetrical care in urban and rural communities? Are midwives, on the whole, better and safer obstetrical attendants in the United States than physicians? Is either the average medical or midwife attendant in rural superior to those in urban communities? To what degree are the variations in the rates as ascertained influenced by the differences in the ages at which childbearing occurs, and in the number of pregnancies per woman, in urban and rural communities? Are so-called native-born women worse or better childbed risks than foreign-born women, and if so, why, and how do the latter vary in this regard among the various immigrant stocks? Are women living in rural districts inherently better obstetrical risks than women of the same race in cities, and are the lower rates obtaining in New York, Philadelphia, and Baltimore due to superior obstetrical care operating on material even less favorable than in the rest of the birth registration area? What are the most important preventable causes of stillbirth? These are some of the many important questions demanding answers.

Prevention and control of illness and death of mother and child are among the most neglected and potentially the most fruitful domains of American public health administration, and, of the problems concerned, the two greatest are the toxemias of pregnancy (including albuminuria and eclampsia) and puerperal fever, of which, the latter is the more readily approached.

My present purpose, however, of calling attention to the importance both to public health administrators and to scientific investigators of specific morbidity and mortality rates in general, and in the puerperal state in particular, of the use of a method of greater exactness than any in common use, is sufficiently served by the material presented.

To those whose duty it is to ascertain the causes and to apply the remedies, it may with propriety be pointed out that vital statistics holds the same relation to advances in public health knowledge and administration that pathological anatomy bears to the advance of fundamental knowledge in clinical medicine and surgery. In investigating the causes of death in the puerperal state, with but comparatively slight increase in work, in municipal and state health departments rates still more refined may be obtained by making subclasses under some of the rubrics—as for instance, in 134, accidents of pregnancy, certain deaths not concerned in childbirth could be eliminated. In administrative efforts directed toward reducing the mortality in the puerperal, including the pregnant, state, deaths from not only puerperal infection, but causes under all other rubrics, must be analyzed, both as to the environment and as to the obstetrical personel under which they occur in different kinds of women. It is likely that at least in the United States, the personal attendant is generally the most important factor. Those physicians and midwives who have consistently high maternal or infantile death rates would become evident, and could be dealt with appropriately. The information acquired in regard to the other causes will suggest suitable remedies.

In all such studies, it is of the greatest importance that careful consideration should be given to race, and by race is here meant race stock and not the deceitful classification by nationality as expressed by geography. If in a large city, the birth certificate be made to show the age of the mother and the number of previous children born live and dead, sufficient data would be accumulated in a short time to determine the relation of the number of children born, both singly and plurally, and the age of mothers to maternal and infantile mortalities. There is happily in the department of health of the city of New York evidence of a direct relation between the statistical studies in the Bureau of Records and the successful efforts toward the administrative control of puerperal fever, but in this respect New York stands in lonely isolation.

Among the lessons disclosed in this study is the deplorable lack of proper appreciation and use of statistical methods and of inductive reasoning from data elicited thereby, in the city and state health departments of the United States birth registration area. It is almost inconceivable that a community large or small, inhabited by people of average intelligence and of relatively great wealth, would fail to apply remedies for such glaring and wholesale women murder, as the data for the United States birth registration area, properly studied, disclose. When the horror of the conditions these data divulge is fully appreciated, it is much to be feared that as usual, the *opinions* of experts, mostly self-appointed, will be followed, with effects disastrous in life and money. The methods to be followed should be based not upon mere opinions drawn from hasty assumptions snatched from the clouds, but upon generalizations reached through inductions from adequate data analyzed, with the aid of appropriate statistical methods, by persons capable of consecutive thought.

Summary.

It has been shown in this investigation that:

1. By using the sum of the living and the still births reported and the number of the deaths of women from causes connected with the puerperal state, rates are calculated expressing the actual risk of dying for women exposed to childbirth with a fair degree of specificity and much more accurately than those obtained by the methods in common use.

2. When this method is applied to the data for births and maternal deaths in the birth registration area of the United States for 1918, the total rate and the rates for causes under the important rubrics are conspicuously high. The total rate is more than double that for England and Wales calculated on live births alone, and this is due to the great excess in the United States' rates for puerperal albuminuria and convulsions and septicemia, and accidents of pregnancy and labor.

3. In the birth registration area of the United States for 1918, the total rate in urban communities was considerably greater (10 per cent.) than that in rural communities.

4. In the four states, Kentucky, Maryland, North Carolina, and Virginia, and in the cities, Washington, D. C., and Baltimore, Maryland the total rates and the rates for the most important rubrics are much higher for negro than for white women, and the negro rates are markedly in excess of the whole in both city and country. The rates for both white and negro women are higher in urban than in rural communities. The excess in total rates, in relation to both race and location, is due chiefly to higher rates for puerperal albuminuria and convulsions and septicemia.

5. As determined for the birth registration area of the United States for 1918, the age of mothers exerts a marked and progressive influence upon maternal mortality from causes connected with child-bearing.

6. As compared with Birmingham, England, and with Stockholm, Sweden, the maternal risk-rate in childbearing is much higher in New York, Philadelphia, Baltimore, Boston, and Washington, D. C., and in the order named. The total rates in these American cities are lower than the urban and rural total rates for the birth registration area of the United States for 1918.

7. The proportion of stillbirths to total births varies considerably in different places and in different races. In the birth registration area of the United States, the proportion for urban and rural communities is approximately the same, and the proportion for the negro is double that for the white, and for the urban negro somewhat greater than for the rural negro.

8. By intensive analysis of the data available in municipal and state health departments, it would be possible to determine with considerable accuracy to what degree some of the causes of high stillbirth rates and most of the causes of high maternal death rates in childbearing are due to poor obstetrical service on the part of physicians and midwives, the extent to which particular individuals are repsonsible for these fatalities, and the measures necessary to reduce them.

REFERENCES.

1. Hendricks, Frederick.
1862. On the vital statistics of Sweden from 1749–1855. Journal of the Statistical Society of London, XXV, 119–174.
2. Dublin, Louis I.
1918. Mortality among women from causes incidental to childbearing. American Journal of Obstetrics and Diseases of Women and Children, LXXVIII, No. 1.

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THE ACTION OF LIGHT ON THE LEUCOCYTE COUNT.

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It has been generally supposed that light produces some effect on the blood, but the experiments which have been done to test this point are many of them unsatisfactory, and the results reported are inconclusive.

In speaking of light one includes, not only the visible spectrum, from 400 to 760 $\mu\mu$, but also the ultra-violet region from 100 to 400 $\mu\mu$, and the infra-red of wave-length longer than the visible. The ultra-violet should be divided into two parts; the near ultra-violet, i.e., the wave-lengths just below the visible, from 300 to 400 $\mu\mu$, and the far ultra- violet, from 100 to 300 $\mu\mu$. The physiological effects produced by these two regions are strikingly different. In previous work practically no attempt has been made to study different wave-lengths of light separately. Experiments have usually been made with sunlight which extends only to 291 $\mu\mu$ in the ultra-violet, and includes, therefore, near ultra-violet and visible light and a large amount of infra-red. Owing to the absorption of water vapour in the atmosphere, very little infra-red of wave-length longer than 400 $\mu\mu$ is transmitted.

The effect produced by light on the erythrocyte count has been found in general to be negative. Long-continued absence of light certainly produces no marked change in the number of red cells. Blessing (1), who acted as physician to Nansen during his expedition in the Fram, published a report in 1898, showing that members of the party exhibited no evidence of anemia during the trip. Similar results were reported for animals by Grober and Sempell (2) in 1919. They examined horses that had worked for years in coal mines, and found no anemia in any case where a satisfactory nutritive condition existed. Borissow (3) kept rabbits and dogs in the light and the dark and reported negative results on the erythrocyte count.

A number of investigators, however, have performed similar experiments with positive results. Graffenberger (4) in 1893 reported that rabbits showed an increase in the percentage of hemoglobin in the light and a decrease in the dark. This result was confirmed by Marti (5) in 1897. He found that rats kept in the dark showed a decrease in the red count and in the percentage of hemoglobin, while those exposed to sunlight, in the day, and an arc light at night, had an increased count. Orum (6) reports similar results, although he introduces a new factor in that he lays emphasis upon changes in blood volume caused by light. Exposure to darkness or to red light causes an initial decrease in the red count and the percentage of hemoglobin, but after a month or two the count and percentage of hemoglobin are increased, owing to a marked concentration of the blood. In white light, and especially in blue light, there is, on the contrary, a condition of plethora, together with an increase in the total hemoglobin. It would seem to be highly desirable to have these experiments repeated with more modern methods of determining the blood volume. If the effects on volume of long continued exposures to red and violet lights are as striking as described by Orum, they would indicate an actual decrease in red corpuscles in the dark (or red), and an increase in the white (or violet), compensated more or less in each case by a diminished or an increased volume of the blood.

All the positive results reported agree and give considerable evidence for believing that there is a decrease in the red count and in the percentage of hemoglobin in the dark, and an increase in the light. These changes, however, are not permanent and the blood count later returns to normal.

The leucocytes show a greater and more definite response to short exposures to light than the erythrocytes. Polito (7) exposed rabbits to direct sunlight for periods of time ranging from fifteen minutes to an hour. The length of exposure made practically no difference, and he reports an increase in the leucocyte count immediately after exposure, amounting to from 2,000 to 5,000 in the total count, with most of the increase in the mononuclear cells. After an hour the blood returned to normal, and unfortunately was not studied further. These animals were exposed to direct sunlight at a temperature of 38° to 42° C., so that the light effect was seriously complicated by a possible heat effect. Murphy (8) has shown that five minutes' exposure of an animal to dry heat, at 55° to 65° C., will produce a rise in lymphocytes of 100 to 200 per cent. over the normal count.

Aschenheim (9) reported that an exposure of infants to direct sunlight, for an hour, usually brought about an increase in the number of leucocytes, the neutrophiles being relatively diminished and the lymphocytes relatively increased.

In 1919 a paper was published by Taylor (10) on the effect of exposure to the sun on the circulating lymphocytes in man. Thirty-eight people were examined before and after a summer at Woods Hole, Massachusetts. In twenty-five of the thirty-eight there was an appreciable increase, both percentage and absolute, in the number of circulating lymphocytes. The differential counts showed an average increase from 27.4 to 35.6 per cent. Eight subjects showed a decrease and five no appreciable change. Of these six failed to tan and three were very dark to begin with. It seems hardly justifiable to attribute this increase solely to ultra-violet light when other agencies were active, such as change in climate and mode of life

It is, however, interesting in this connection to find that the blood counts made by Chamberlain, Vedder, Phalen, Wickline and others, on Americans in the Philippines and on native Philippinos, show an increase in the percentage of lymphocytes and a decrease in polymorphonuclears, as a result of residence in the tropics. Their results have been summarized briefly in the following table, from Chamberlain and Vedder (11) (see Table I).

TABLE I.

Observer.	Race.	Number observed.	Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Total count.
Wickline (12).....	Americans, 18 months' service	104	54.9	33.4	6.1	6,831
Guerrera (13) and Sevilla..........	Philippinos	129	51.6	34.5	4.1	—
Phalen (14)........	Americans, more than one year's service	115	58.7	32.6	4.6	—
Chamberlain (15) .	Igorots	40	46.9	37.2	5.7	—
Chamberlain (11) and Vedder.....	Americans, 14 months' service	72	56.8	31.7	6.9	7,304
Chamberlain (11) and Vedder.....	Philippinos	50	52.2	29.9	6.6	9,248
Simon (16).......	Normal count, temperate climates	—	60 to 70	20 to 30	1 to 6	5,000 to 10,000

Wickline (12) has shown that these low polymorphonuclear and high lymphocyte counts, for Americans living in the Philippines, developed gradually. He made counts on 104 soldiers, on arrival and 3, 12, and 19 months later. After 3 months the mononuclear cells averaged 31.4 per cent., after 12 months 34.4 per cent., and after 19 months 39.5 per cent. The small mononuclears, which undoubtedly correspond to the small lymphocytes, give a percentage of 21.8, 26.6, and 33.4 at the three examinations. During this change the total count stayed nearly constant, being 6,943 at the preliminary examination, and 6,699, 6,624, and 6,831 after 3, 12, and 19 months, respectively. How much of this effect is due to the action of tropical light is impossible to say. There has been a very prevalent idea that the solar spectrum extends further into the ultra-violet in the tropics. This is not true. The solar spectrum has been photographed at Manila, by means of a Rowland grating, by Freer (17) and was found to extend to 291 $\mu\mu$. Miethe and Lehman (18) made measurements at Assuan, Berlin, Zermatt, Görnergrat and Monte Rosa and found the limit of the spectrum always the same, 291.55 $\mu\mu$ to 291.21 $\mu\mu$. Neither latitude nor altitude thus makes any appreciable difference in the extent of the solar spectrum in the ultra-violet. However, light in the tropics has greater intensity than in temperate zones, so that in this way there may be a greater exposure in the tropics to ultra-violet light, as well as to visible light and heat. Most of the effects produced on soldiers in the tropics, such as increased pulse rate, respiration, and body temperature, can also be produced by moist heat. (Phalen 14.) Whether the blood changes are due to this cause, or whether they result from an increased exposure to ultra-violet light, is at present impossible to say.

In general, however, one can conclude from former work that exposure to sunlight results in an increase in lymphocytes. The experiments have all been complicated by the action of heat and are suggestive rather than conclusive.

In the experiments reported here an attempt was made to study separately the effects of different regions of visible and ultra-violet light, using short exposures.

METHOD.

Rabbits were exposed to the light of an iron arc for one hour, at a distance of ten inches. At this distance there was no heat effect, the temperature remaining below 25° C. The rabbits were enclosed in a box, with only the head exposed, and the eyes were covered during exposure, so that only the ears were radiated. The hair was clipped from the ears before radiation, so that the skin was well exposed. Blood counts, both total and differential, were made before and after exposure, and the animals were then returned to a basement room, very dimly lighted by diffuse light, in which all animals had been kept some weeks or months before being used. The blood was counted daily until a normal condition seemed to be reestablished. This usually occurred in about three weeks.

The iron arc gives a brilliant light which, when examined spectroscopically, is so rich in lines that it almost amounts to a continuous light source. It reaches to about 238 μμ, and is strong throughout the ultra-violet. No quantitative work has been done on the relative intensity of the iron arc throughout the spectrum. Pflüger (19) investigated the energy distribution in the spark spectrum of iron, with a bolometer, and found the greatest energy in the ultra-violet. The distribution in the arc is very different from that in the spark, but it can be assumed that the ultra-violet region is at least as intense as the visible, and probably of greater intensity. In Fig. 1 the distribution of energy in the solar spectrum is given, showing that in the sunlight there is a great exposure to visible light and a very weak exposure to ultra-violet, while the reverse is true of the iron arc.

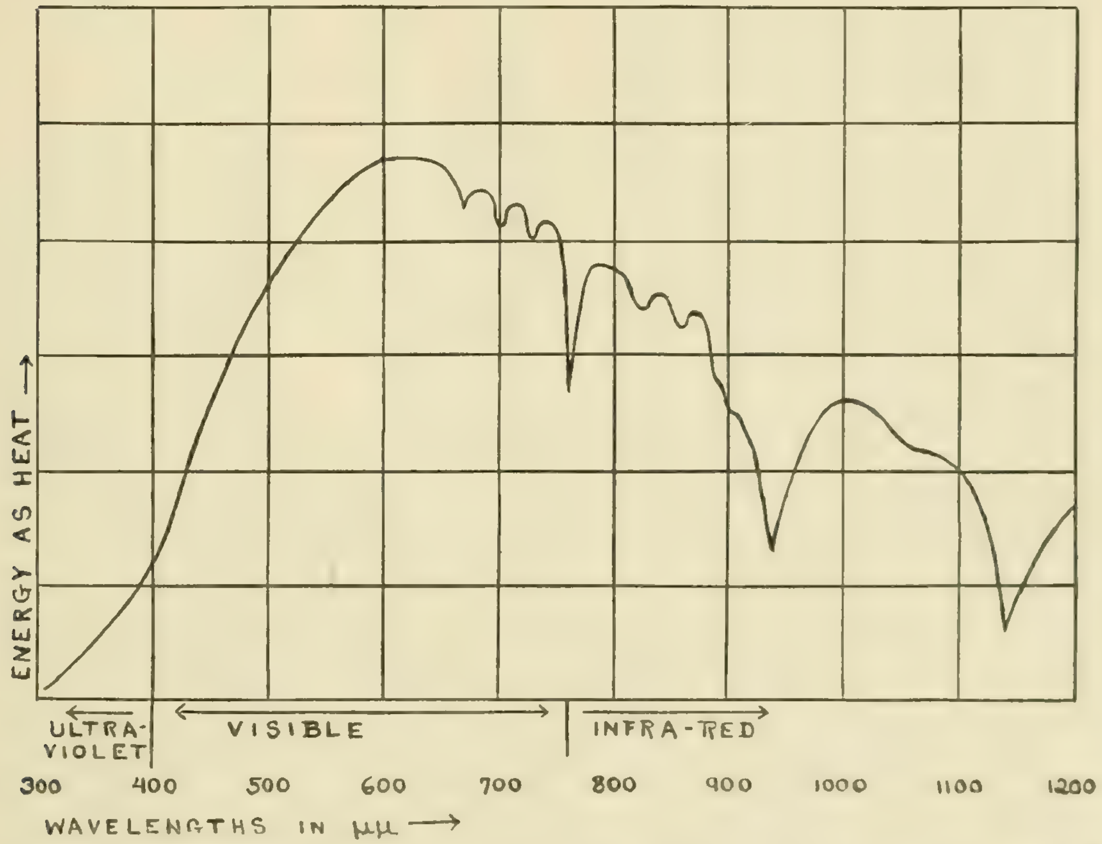

FIG. 1. Distribution of energy in the solar spectrum (Langley). Ordinates —energy as heat, units arbitrary. Abscissae—wave-lengths in μμ.

In order to investigate separate regions of light a number of different filters were used. The absorption of these is well known, but the particular filters were tested by photographing the transmitted light with a meter focus concave grating spectrograph. The transmission of these filters is given in Table II.

TABLE II.

Source.	Transmission ($\mu\mu$).
Iron arc unscreened	238 →
Iron arc through glass	320 →
Iron arc through ultra-violet glass*	330 → 390
Iron arc through picric acid	450 → 750
Iron arc through red glass	650 →

It is impossible to isolate the ultra-violet below 300 $\mu\mu$ by means of a filter, as this region is strongly absorbed by all substances except those, such as quartz or fluorite, which also transmit the visible. By comparing the effect of the radiation with and without the plain glass screen it is possible to estimate which part of the effect is due to the far ultra-violet below 300 $\mu\mu$.

The absorption of blood is compared with the transmission of the filters in Fig. 2. It might be expected that the effect of light on the blood would be due to the absorption of light energy by some constituent of the blood, with resultant physical and chemical action. It can be seen that the region from 230 to 300 $\mu\mu$ is absorbed by the hemoglobin and also by the blood serum. The hemoglobin also absorbs the region from 300 to 450 $\mu\mu$, and has two absorption bands in the yellow, between 530 and 590 $\mu\mu$.

It is impossible to say anything very definite in regard to the absorption of the skin, although the experiments reviewed by Jesionek (20) show roughly that light of wave-length shorter than 300 $\mu\mu$ is absorbed by the epidermis, and that bloodless tissue will transmit some rays throughout the visible.

TECHNIQUE OF BLOOD COUNTING.

A word should be said about the technique of blood counting used in this work. Previous work, by the author, on rabbits had proved unsatisfactory, owing to large and inexplicable variations in the white count, although the red counts had been reasonably constant.

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* This ultra-violet glass was obtained from Dr. R. W. Wood. It absorbs the visible and transmits the near ultra-violet, just below the visible.

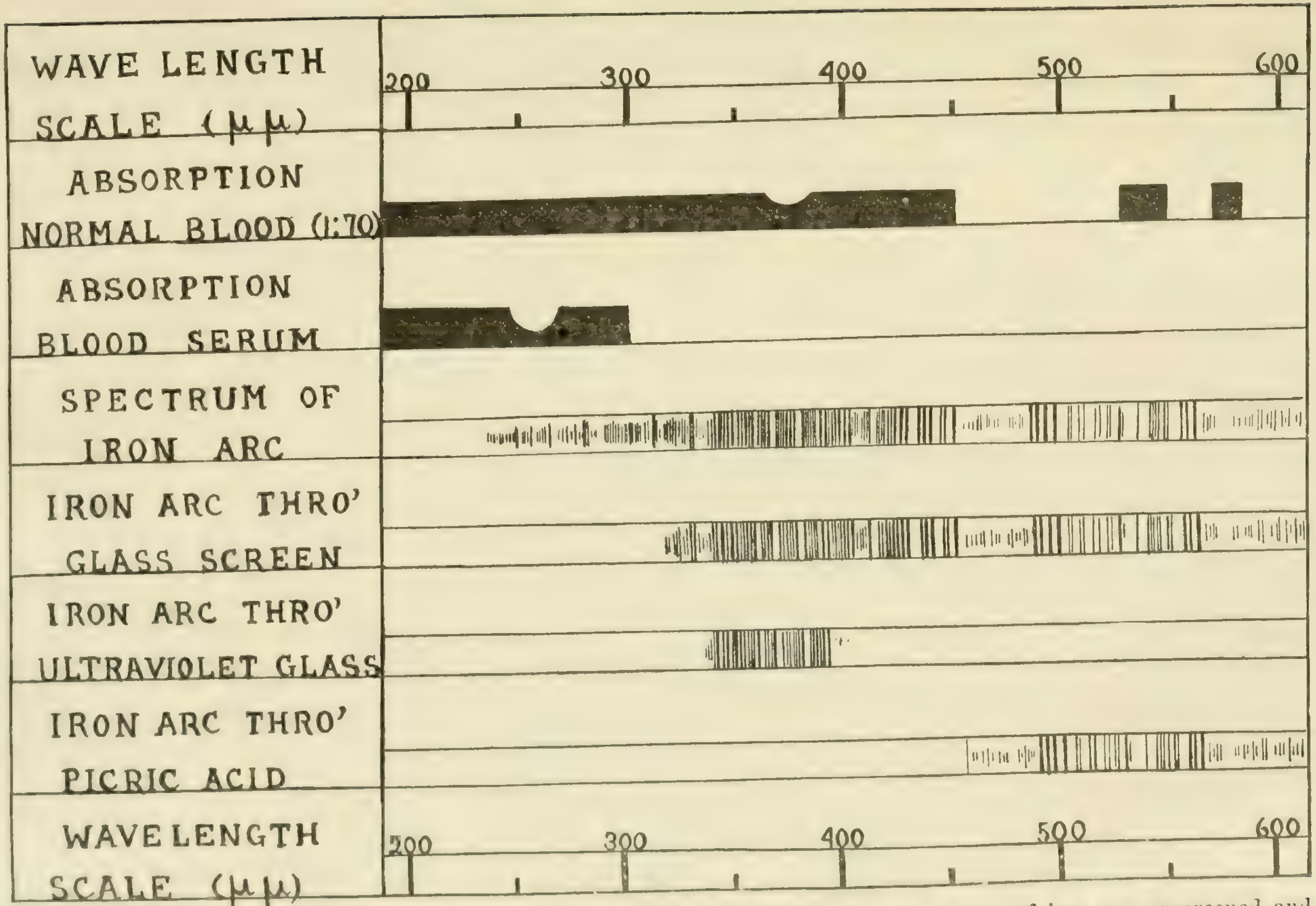

FIG. 2. Absorption spectra of blood and blood serum compared with emission spectra of iron arc unscreened and iron arc through filters.

Believing this to be due to different conditions of circulation in the ear when the counts were made, the leucocyte counts on the normal, untreated ear, and the ear when well flushed with hot water (45° C.) were compared. The results obtained are shown in Table III.

TABLE III.

Leucocyte Counts.

	Untreated.	Flushed once.	Flushed twice.
Rabbit I	16,640	11,700	—
Rabbit II	16,930	12,510	11,900

Apparently, on flushing the ear and getting a rapid circulation through it, the white count drops to a steady level, which is presumably that of the circulating blood. By following this technique very consistent counts were obtained. Some of the rabbits used gave very constant counts from day to day, when not subject to radiation. Others showed variations, and these responded to radiation with larger changes than the rabbits with constant counts, although the changes were of the same type.

In making the differential count the smears were stained with Jenner's stain and the following classification was followed; large mononuclears (cells in which the ratio of nucleus to cystoplasm is relatively small); lymphocytes (all cells, both large and small, in which this ratio is relatively large); polymorphonuclears (these are called pseudo-eosinophiles by Klieneberger and Carl (21), and correspond to the neutrophiles in man); eosinophiles, and mast cells. A normal differential count, as given by the non-radiated rabbits, is roughly as follows:

	Large mononuclears.	Lymphocytes.	Polymorphonuclears.	Eosinophiles.	Mast.
Per cent	1	64	30	1	4

RESULTS.

In the first experiments the total red and total white counts were followed for each animal, but no differentials were made. The erythrocyte count gave negative results. The leucocyte count, however, showed marked and consistent variations after exposure to light.

The red count was therefore abandoned, and total and differential white counts were made throughout the rest of the work.

RESULTS OF EXPOSURE TO THE UNSCREENED IRON ARC (FIGS. 3, 4 and 5).

Immediately after the exposure there is a sharp drop in the total count (Fig. 3). This is contrary to Polito's results (7), but the conditions of the experiment were also different from his. The differential (Fig. 4) shows this to be due entirely to a drop in the number of lymphocytes, so that it cannot be attributed to an increase in blood volume. By the next day the total count (Fig. 3) was approximately normal, and then rose steadily reaching a maximum on the fifth day. In the first experiments there was a sharp rise above normal on the second day. This was not marked in later experiments, due perhaps to the fact that the same animals were used repeatedly, and thereby

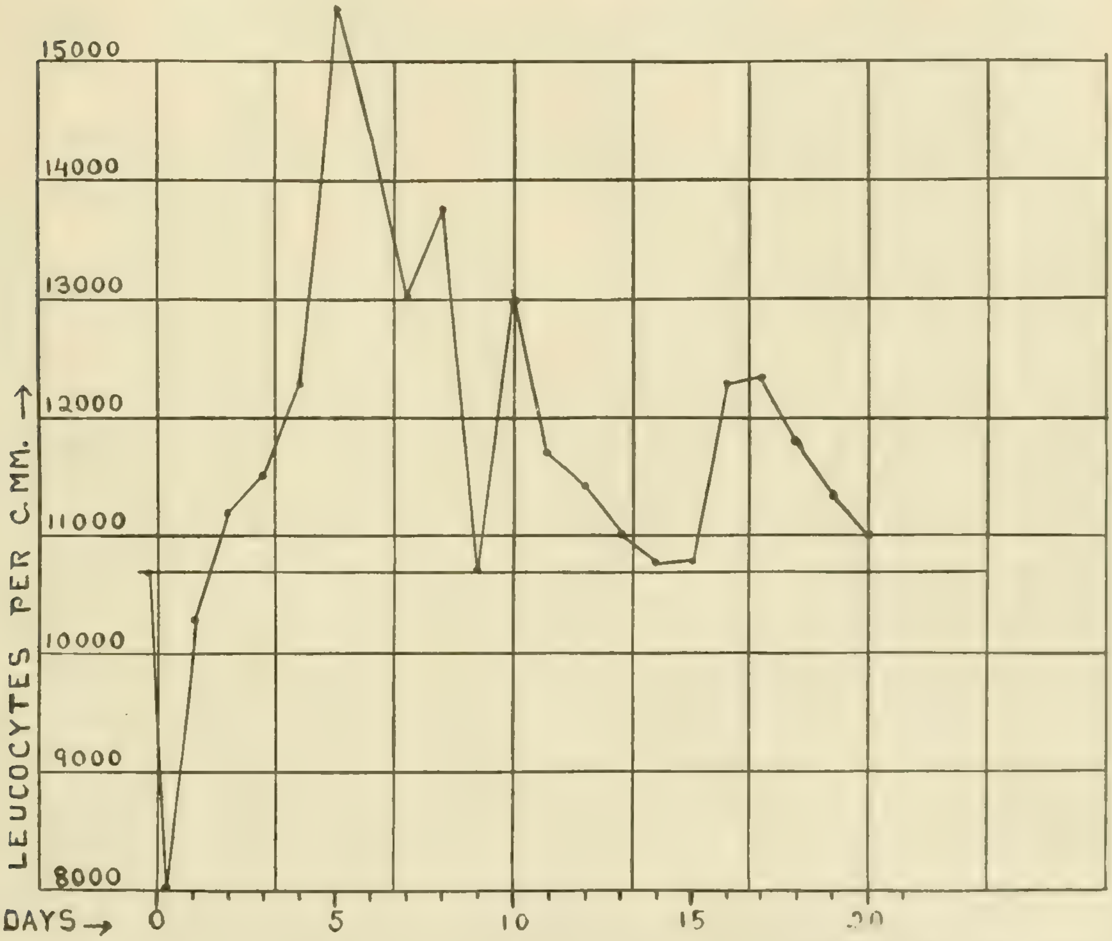

FIG. 3. Total leucocyte count, after exposure to unscreened iron arc (average of five rabbits). Ordinates—leucocytes per c.mm. of blood. Abscissae—time, in days, after exposure.

lost their susceptibility somewhat. The differential curve (Fig. 4), however, shows a rise in lymphocytes on the second day after exposure, although it is much less marked than the rise on the fifth day. Comparing the total curve with the differential, the polymorphonuclears are seen to remain nearly steady throughout, though a little below normal, while the total curve and lymphocyte curve run almost parallel. The large maximum on the fifth day is followed by a return to normal on the ninth. This is followed by two small rises on the eleventh and sixteenth days. After three weeks the blood was apparently normal again, so that the experiments were not carried any further. The result of the exposure to the bare iron arc is an approximately normal polymorphonuclear count and, except for the initial drop immediately after exposure, a lymphocyte count above

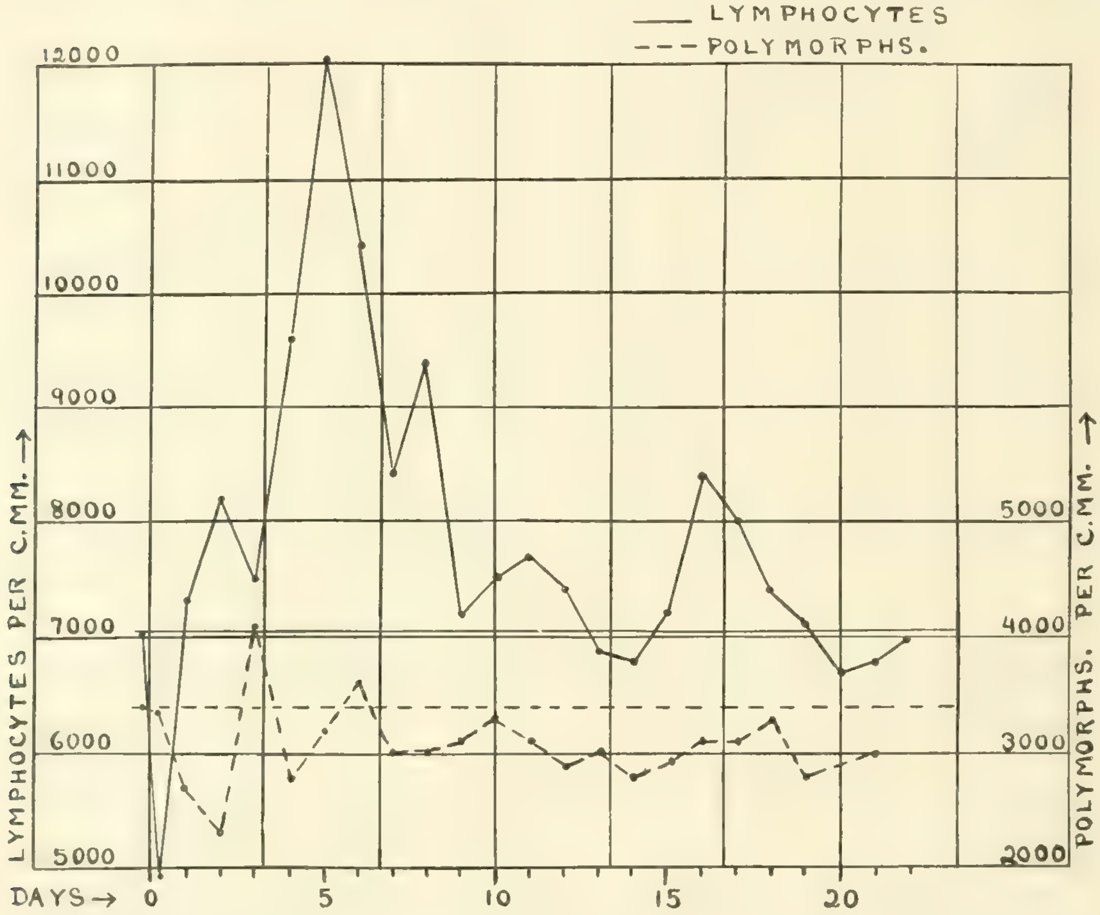

FIG. 4. Lymphocyte and polymorphonuclear counts, after exposure to unscreened iron arc (average of four rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure.
———————— lymphocytes — — — — — — — polymorphonuclear leucocytes.

normal for three weeks. The maximum rise on the fifth day amounts to an increase of almost 100 per cent. over the original count.

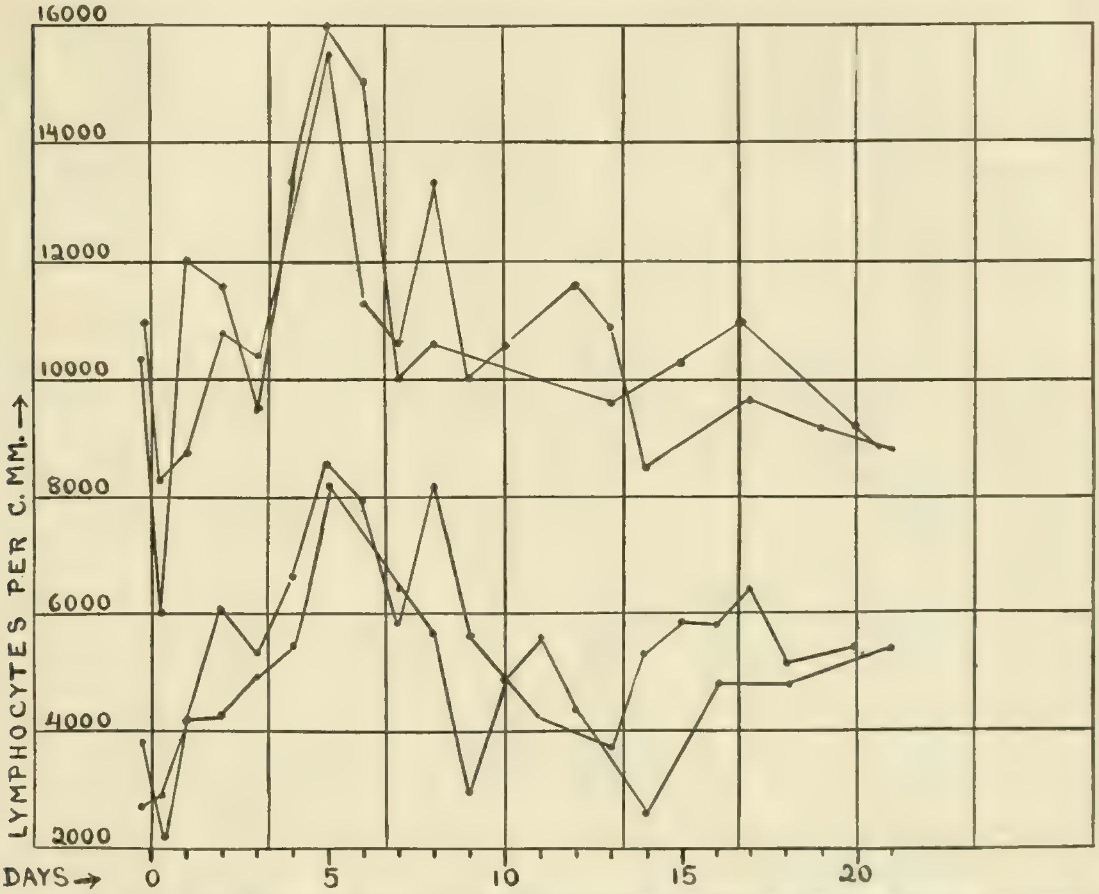

FIG. 5. Lymphocyte counts on four individual rabbits (same rabbits, averaged in Fig. 4), after exposure to unscreened iron arc. Ordinates—lymphocytes per c.mm. of blood. Abscissae—time in days after exposure.

In Fig. 5 the lymphocyte curves of four individual rabbits are plotted, in order to show how closely they agree with each other and with the average lymphocyte curve in Fig. 4.

RESULT OF EXPOSURE TO THE IRON ARC THROUGH A GLASS SCREEN

FIG. 6.

Only two animals were radiated through glass, but the results were sufficiently parallel to warrant drawing conclusions from them. The total leucocyte count and the absolute lymphocyte and polymorphonuclear counts are all plotted in Fig. 6. There is practically no change in the total count immediately after radiation, but the differential count shows a large decrease in lymphocytes, which is balanced by a corresponding increase in polymorphonuclears. The total count falls steadily until the third day, after which it fluctuates until the thirteenth day, although far below normal all the time. On the thirteenth and fourteenth days there is a marked drop, followed by a gradual rise to normal. After the initial rise and fall, immediately following the exposure, the polymorphonuclears stay approximately normal, except for a small rise on the seventh day. The decrease in total count is therefore due entirely to a fall in the number of lymphocytes. These stay far below normal for three weeks or more, and are reduced to one third of the original count on the thirteenth day after exposure.

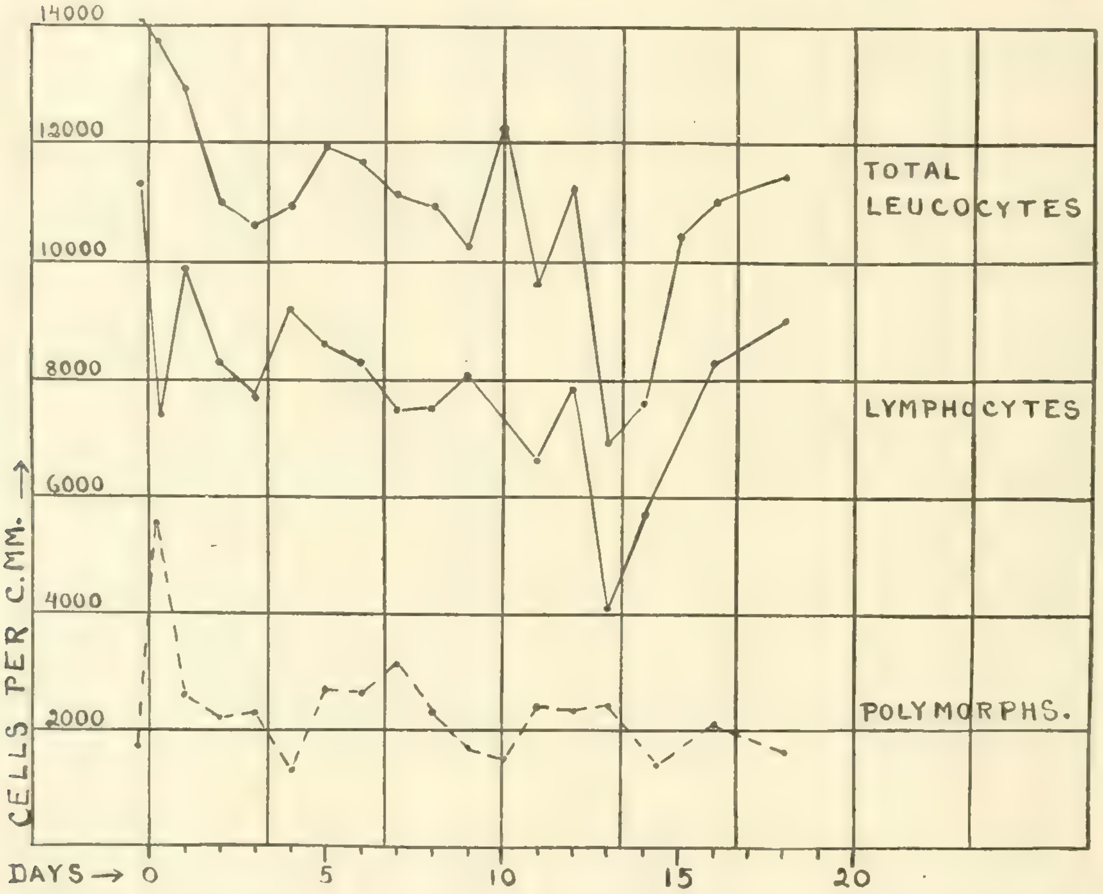

FIG. 6. Total leucocyte, and absolute lymphocyte and polymorphonuclear counts, after exposure to iron arc through glass (average of two rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure——— ——— leucocytes and lymphocytes — — — — — — — — polymorphonuclear leucocytes.

The curves for radiation with the iron arc bare (Figs. 3 and 4), and with the iron arc through glass (Fig. 6), are strikingly different. Neither radiation produces any change in the number of polymorphonuclears after the first day, but both produce a very profound change in the number of lymphocytes. The lymphocyte count is greatly increased by radiation with the bare iron arc while it is decreased by radiation with the iron arc through glass. As the glass transmits everything above 320 $\mu\mu$, one must conclude that the stimulating action is due to the region between 230 and 320 $\mu\mu$; i.e., the far ultra-violet. It is probable that if this region could be isolated and used separately the increase would be much greater as the depressing action of the rest of the spectrum would not have to be counteracted.

RESULT OF EXPOSURE TO THE IRON ARC THROUGH ULTRA-VIOLET GLASS (TRANSMISSION 330 TO 390 $\mu\mu$) (FIGS. 7, 8 AND 9).

In order to further analyze the action of light of wave-length longer than 320 $\mu\mu$, the effect of special regions was investigated. With Wood's ultra-violet glass the region between 330 and 390 $\mu\mu$ was tried. These experiments gave remarkably consistent results.

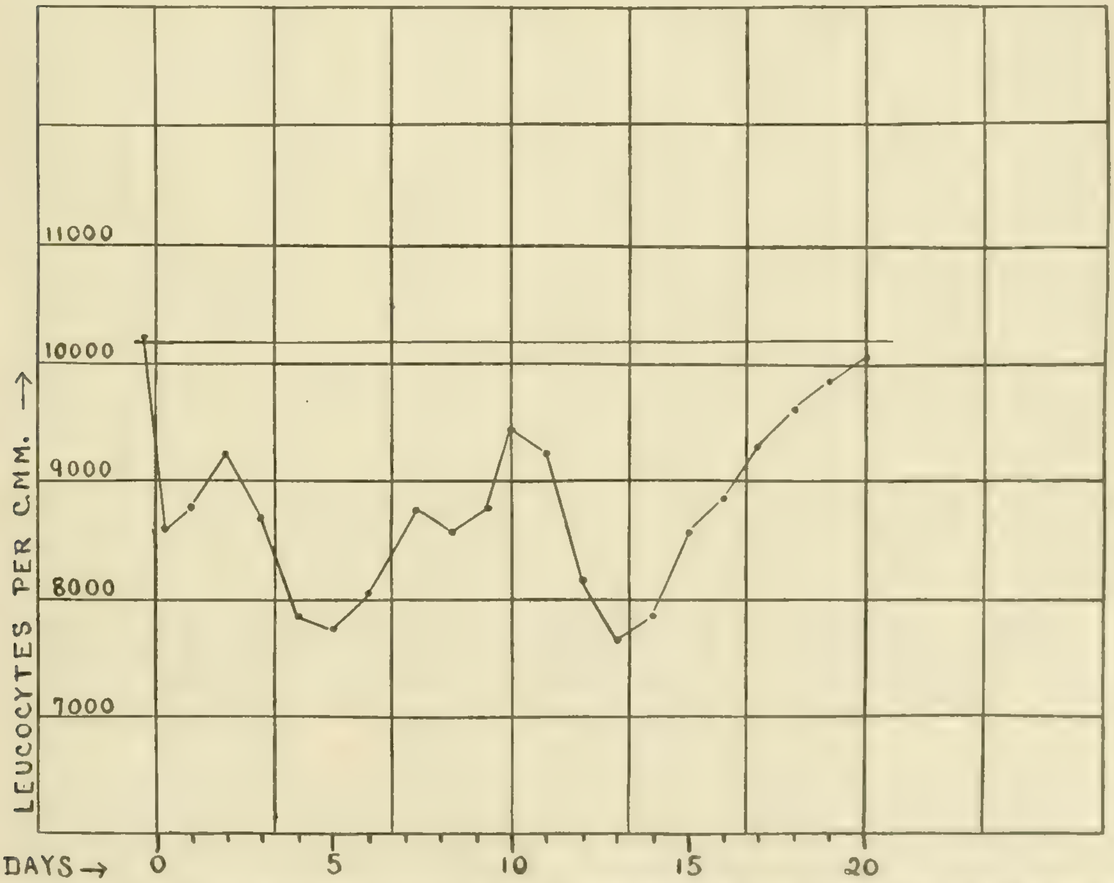

FIG. 7. Total leucocyte count, after exposure to iron arc through ultra-violet glass (average of five rabbits). Ordinates—leucocytes per c.mm. blood. Abscissae—time, in days, after exposure.

The total count (Fig. 7) shows a fall, followed by a rise on the second day. This is followed by a fall on the fifth day, a gradual rise on the tenth, another fall on the thirteenth and a gradual rise to normal. The differential (Fig. 8) shows that the initial fall is due entirely to lymphocytes, as the polymorphonuclears rise slightly. The rise on the second day is due to lymphocytes alone, but the two falls on the fifth and thirteenth days are due to both polymorphonuclears and lymphocytes. This region therefore causes a decrease in both polymorphonuclears and lymphocytes, but especially in the latter. Although the curves are not analogous throughout, the lymphocyte curve for radiation through ultra-violet glass (Fig. 8) is somewhat similar to that for radiation through plain glass (Fig. 4), especially as regards the large drop on the thirteenth day. This indicates that

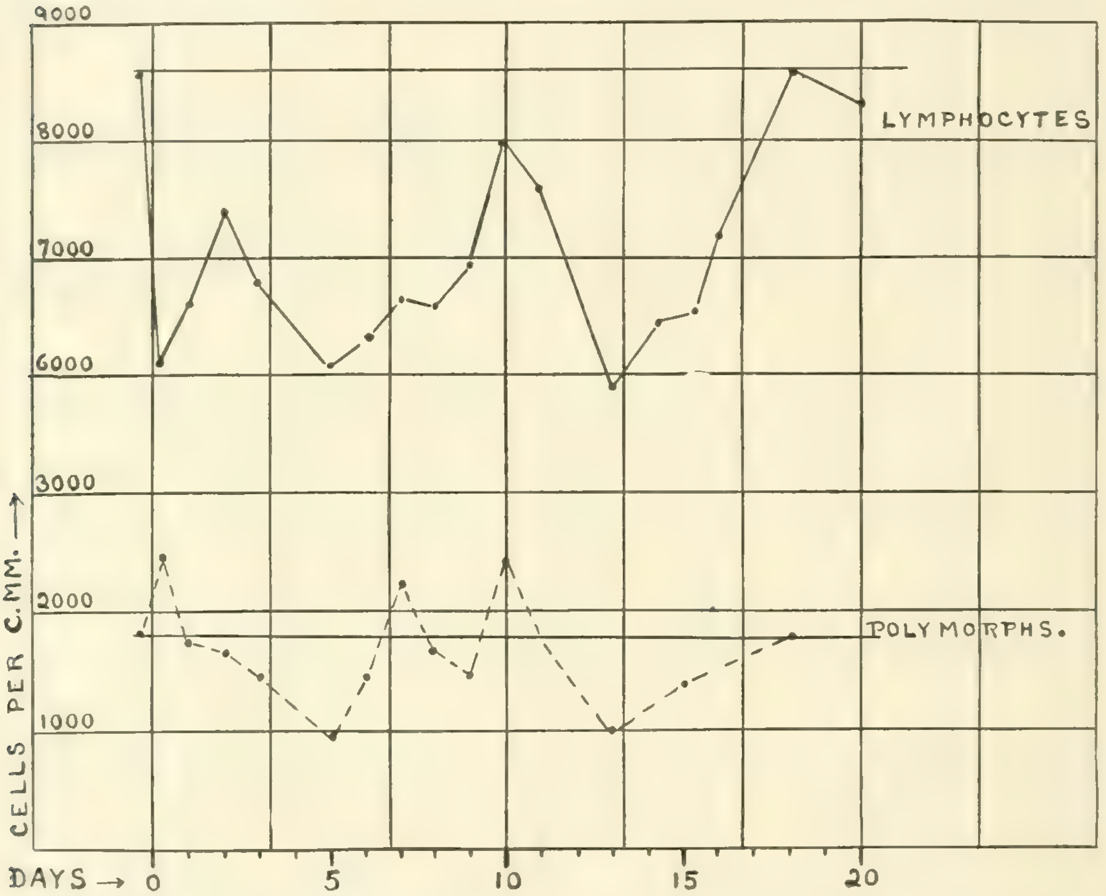

FIG. 8. Lymphocyte and polymorphonuclear counts, after exposure to iron arc through ultra-violet glass (average of three rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure ——————— lymphocytes — — — — — — — — polymorphonuclear leucocytes.

the depressing action of the radiation through glass is largely due to the near ultra-violet in this radiation, which is not surprising as the iron arc is very rich in lines in this region.

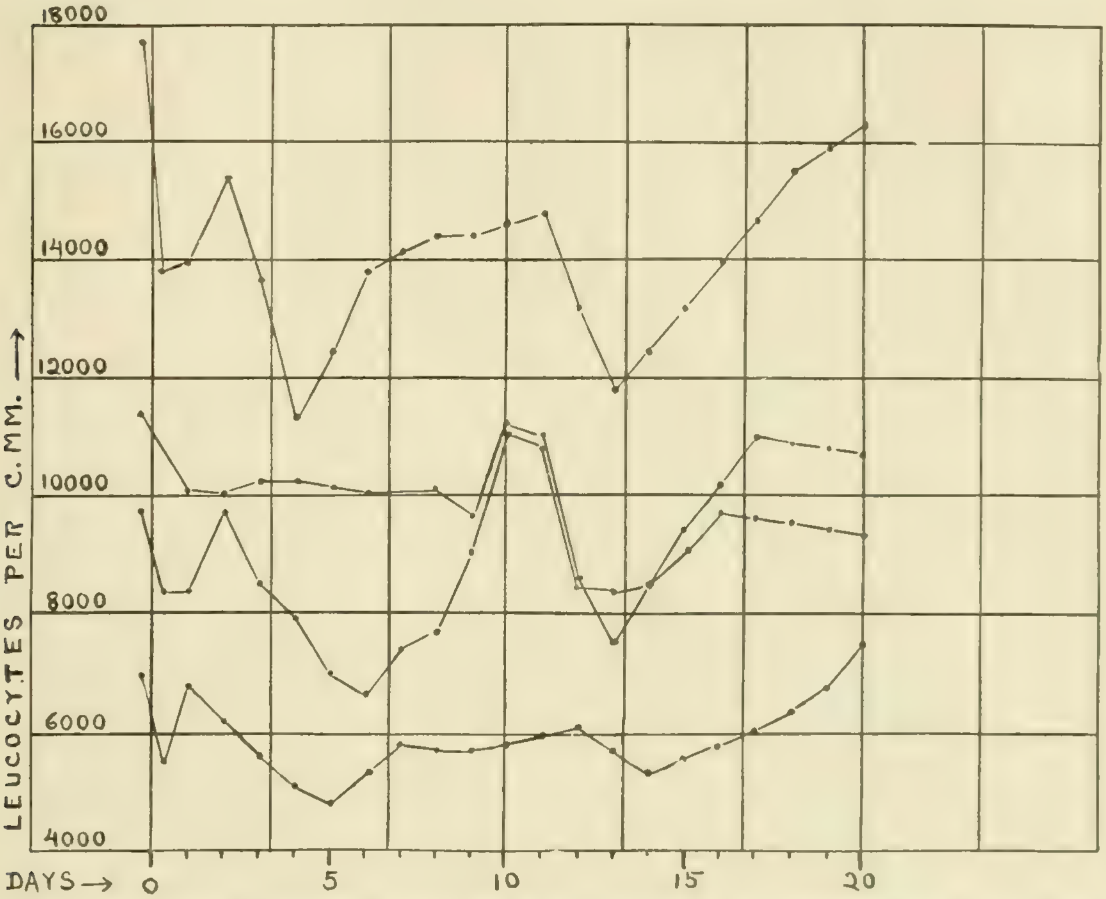

FIG. 9. Total leucocyte counts on four individual rabbits, after exposure to iron arc through ultra-violet glass. Ordinates—leucocytes per c.mm. of blood. Abscissae—time, in days, after exposure.

Fig. 9, in which the total white counts of four individual rabbits are plotted, shows how closely they agree with each other and with the average curve.

RESULT OF RADIATION WITH THE IRON ARC THROUGH A PICRIC ACID SCREEN (TRANSMISSION 450 → 750 $\mu\mu$) (FIG. 10).

The region transmitted by this screen includes the two yellow absorption bands of hemoglobin.

There is a moderate drop in the total count immediately after radiation, due to the combination of a large drop in lymphocytes and a small rise in polymorphonuclears. After this immediate effect the total count, the lymphocyte and the polymorphonuclear counts follow parallel curves, showing a large rise on the first and second days after radiation, followed by a sharp drop on the third, an equally sharp rise on the fourth, and a return to normal on the seventh, after which no noticeable variation was found.

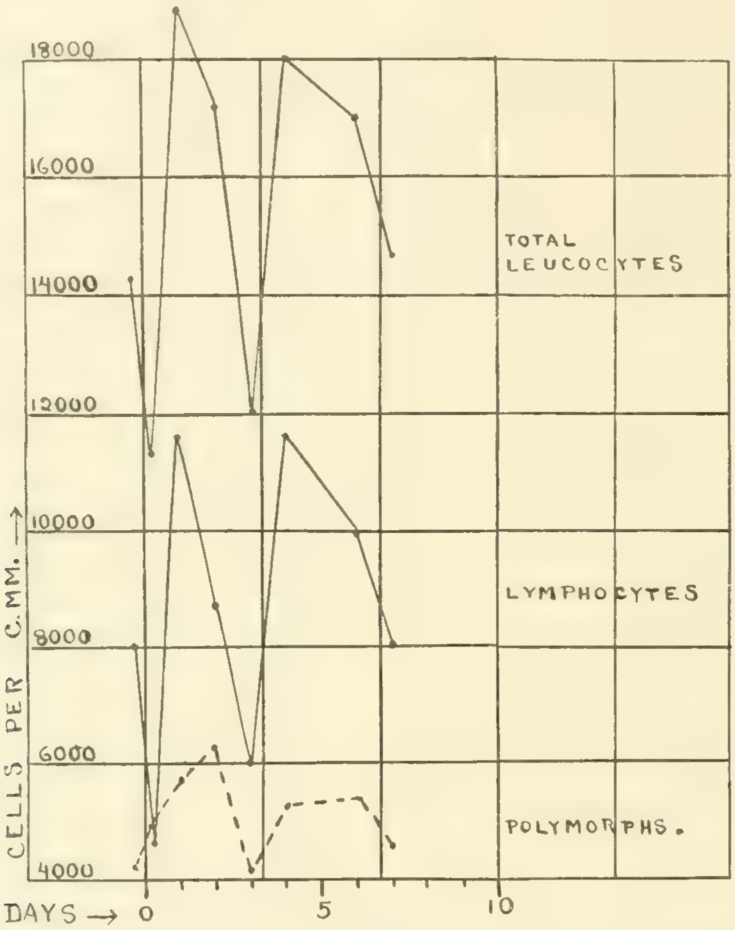

Fig. 10. Total leucocyte and absolute lymphocyte and polymorphonuclear counts, after exposure to iron arc through picric acid screen (average of two rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure ——————— leucocytes and lymphocytes — — — — — — — polymorphonuclear leucocytes.

Result of radiation with iron arc through red glass (Fig. 11).

This resulted in an immediate fall in lymphocytes which returned to normal by the second day, and no further change was then observed. The polymorphonuclears were practically unchanged. One may conclude, therefore, that radiation of wave length longer than 650 $\mu\mu$ produces no effect other than the usual lymphocyte drop immediately after radiation.

Since there is always a drop in lymphocytes immediately after radiation, irrespective of the wave-lengths used, it seemed possible that this drop might be due to some other agency than light. A series of control experiments was performed on the animal to see whether the mere position in the box might be responsible for this drop. They were placed in the box for an hour, and blood counts were made before and after, which showed no significant change in either total or differential counts.

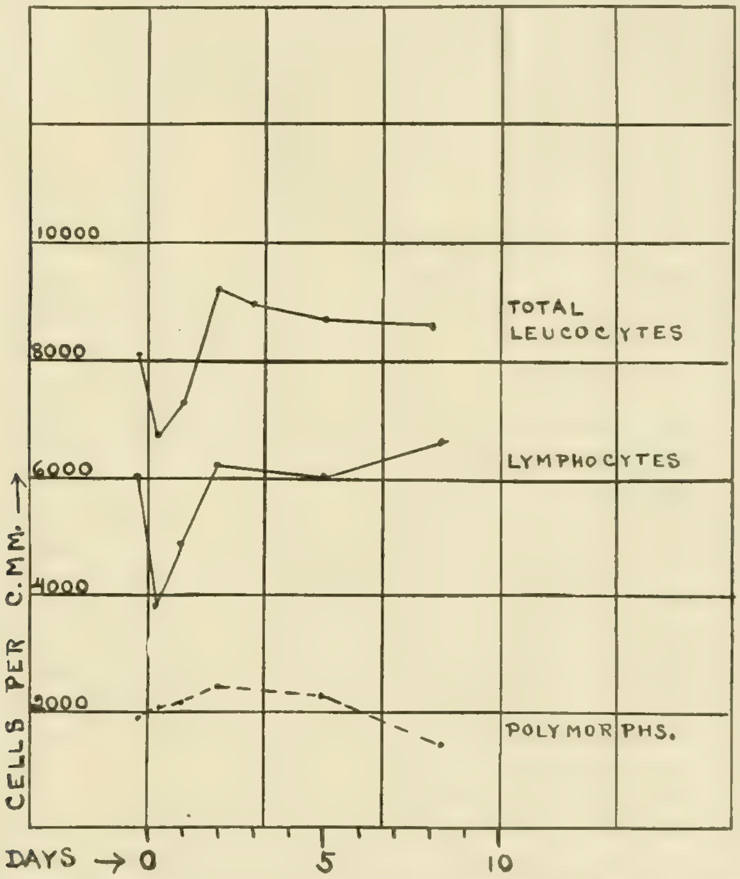

FIG. 11. Total leucocyte and absolute lymphocyte and polymorphonuclear counts, after exposure to iron arc through red glass (average of two rabbits). Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after exposure. ———— leucocytes and lymphocytes — — — — — — polymorphonuclear leucocytes.

EFFECT OF REPEATED RADIATION.

Radiation of one hour daily, for a week, seemed to give no greater response to the bare iron arc than a single radiation. This, however, has not been very extensively tried.

Discussion.

There is always a fall in the number of circulating lymphocytes immediately after exposure to light of any wave-length. When this is accompanied by an increase in polymorphonuclears, as in the radiation through plain glass, the total count is unchanged, but usually it results in a fall in the total count as well. Light of wave-length greater than 650 $\mu\mu$ has no effect except this usual fall in lymphocytes immediately after exposure.

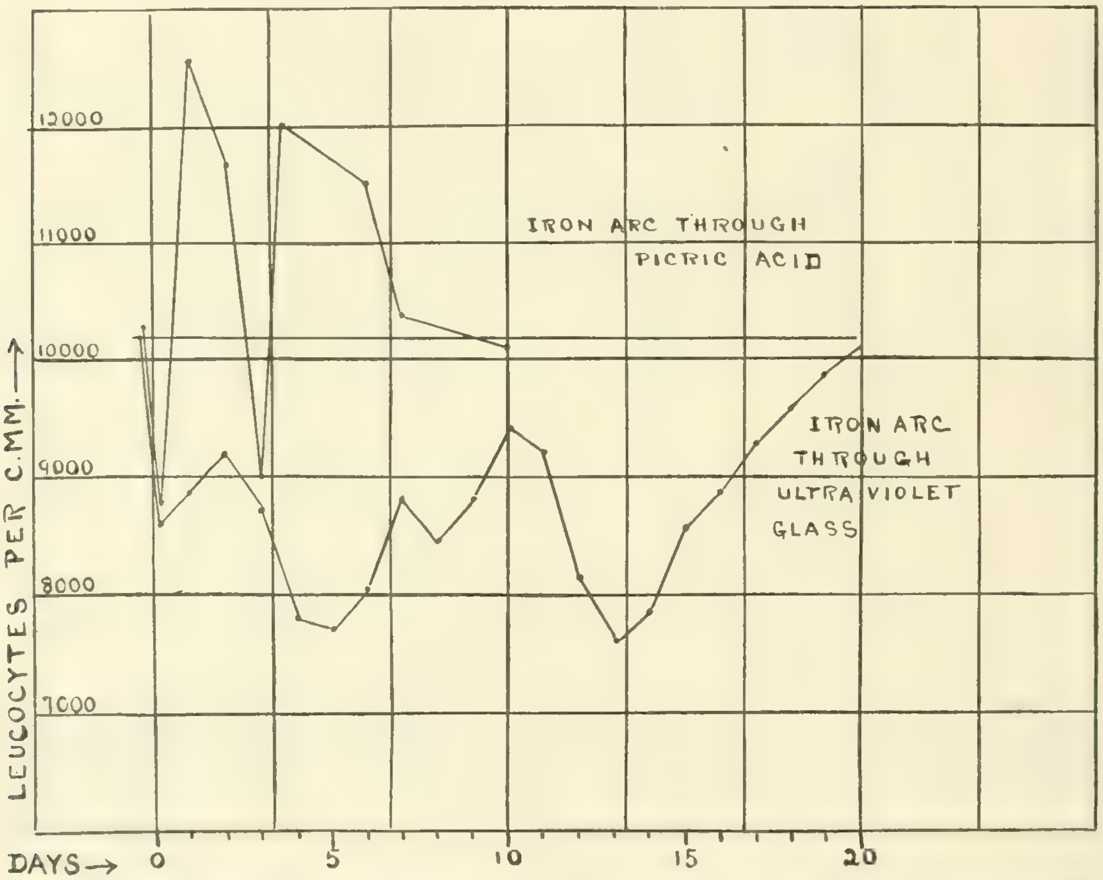

Fig. 12. Average total leucocyte counts, after exposure to iron arc through picric acid and through ultra-violet glass, showing complementary effects. Ordinates—leucocytes per c.mm. of blood. Abscissae—time, in days, after exposure.

Light of wave-length shorter than 320 $\mu\mu$, or perhaps 300 $\mu\mu$, results solely in a great increase in lymphocytes. This confirms the suggestion, made in the previous work referred to, that the ultra-violet stimulates a lymphocytosis, leaving the polymorphnuclears constant in absolute number. It is, however, only the far ultra-violet ($\lambda < 300 \mu\mu$) which is capable of this action, as the region of ultra-violet just below the visible has a depressing effect on the lymphocytes (See Fig. 8). This near ultra-violet gives a rise in polymorphonuclears immediately after exposure, which is also found as a result of radiation through plain glass, but this is followed by a decrease below normal.

The region between 450 and 650 $\mu\mu$, however, increases both the polymorphonuclear and lymphocyte counts. From this fact a sufficient explanation can be found perhaps for the experimental observation that animals kept continually in sunlight do not as a rule show a different leucocyte count from those kept in darkness. The stimulating effect of the yellow light, and the small amount of far ultra-violet present in the solar spectrum, is offset by the depressing action of the blue, violet, and near ultra-violet as indicated in Fig. 12. In the solar spectrum the intensity of yellow light is greater than the intensity of the blue and violet, and the relative intensity is possibly such that this balancing action is practically complete, except when an unusual intensity of far ultra-violet (290 to 300 $\mu\mu$) is present, as may be the case in the tropics. In the spectrum of the iron arc through glass which is composed of somewhat the same range of wave-lengths as the sunlight, although it does not reach as far as 290 $\mu\mu$ in the ultra-violet, the intensity of the violet and near ultra-violet is relatively very much greater, and the total effect is more parallel to that of the near ultra-violet alone (See Fig. 6).

CAUSE OF EFFECT.

The cause of the action of light on blood is not known. Experiments are now in progress on this point, but as yet no satisfactory conclusion has been reached.

The invariable drop noticed in the lymphocytes immediately after radiation, furnished the suggestion that some product of the disintegration of these cells may act as a stimulus to the lymph nodes and thus account for the subsequent rise. This suggestion is not borne out by the results of radiation with the longer wave lengths, 650 $\mu\mu \rightarrow$, but it was tested directly by the following experiments, with a negative result.

An emulsion of lymphocytes in salt solution was made from the lymph glands and thymus gland of one rabbit. Half of this was injected into a second rabbit, and the rest, after being radiated in a quartz tube, with the bare iron arc, for one hour, was injected into a third rabbit. These two rabbits had had their blood counted before injection, and were then examined daily for a week. They both showed a great and immediate increase in polymorphonuclears, due possibly to the introduction of foreign protein. This was accompanied by a decrease in lymphocytes. By the next day the blood was practically normal, and no further significant change was found.

A more probable explanation is that the absorption of light by the hemoglobin or plasma of the blood may lead directly or indirectly to the formation of products that have a stimulating effect on lymphocyte production. In line with this suggestion the following experiments were made.

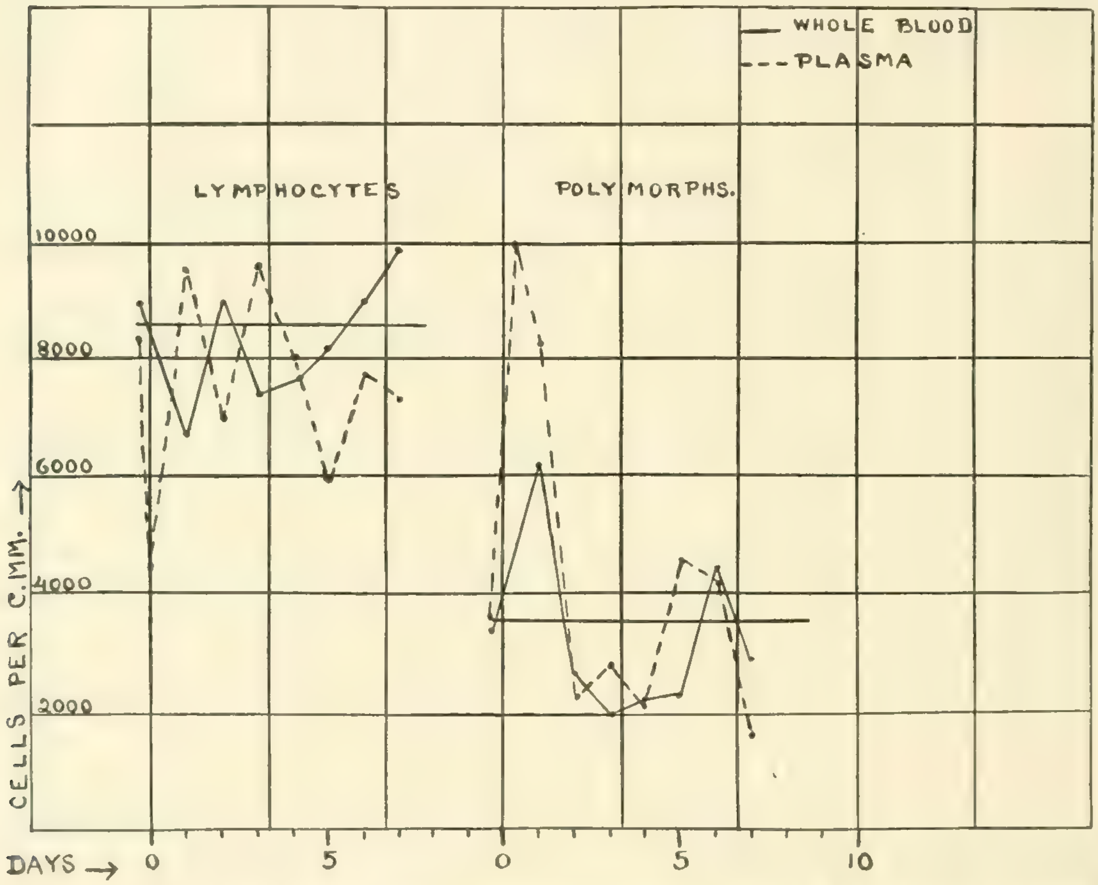

FIG. 13. Lymphocyte and polymorphonuclear counts for rabbits injected with radiated whole blood and radiated plasma. ———————— injected with radiated whole blood — — — — — — — injected with radiated plasma. Ordinates—cells per c.mm. of blood. Abscissae—time, in days, after injection.

Experiment I. 3 c.c. of blood from the heart of a rabbit were mixed with 1.5 c.c. of heparin (20 mgm. in salt solution) to prevent coagulation (22), radiated in a quartz tube with the bare iron arc for one hour, and then injected back into the same animal through an ear vein.

Experiment II. 4 c.c. of blood from the heart of a rabbit were mixed with 2 c.c. of heparin (25 mgm. in salt solution), centrifugalized, and 3.5 c.c. of clear plasma separated. This plasma was radiated for one hour in a quartz tube with the bare iron arc, and was then injected into the same rabbit through an ear vein.

The results are shown in Fig. 13. The total count in the rabbit with injected plasma showed a suggestive rise in the total count on the first day after injection. The differential, however, proved this to be due entirely to a very great rise in polymorphonuclears. Apparently the radiated plasma acted like a foreign protein. Except for this increase in polymorphonuclears the injected radiated plasma had no other stimulating effect.

The injection of radiated whole blood also gave a polymorphonuclear rise, but it was less marked than in the case of the plasma. Except for this change the action was depressing, both on lymphocytes and polymorphonuclears. The stimulating effect of the far ultra-violet on lymphocyte formation is apparently not due to an action resulting directly from the absorption of light by the hemoglobin or plasma, although no general conclusion can be drawn from this single experiment.

As it is known that ultra-violet light exerts a direct effect on the skin, leading to sunburn or marked inflammatory changes, it is possible that its action on this tissue may be responsible indirectly for the lymphocytosis. As bearing upon this suggestion an attempt was made to determine the effects of radiating a bloodless ear. In three rabbits the common carotid artery on one side was exposed under local action of cocaine and clamped. The corresponding ear was then exposed for an hour to the bare iron arc. The experiments were not satisfactory. It was found as a matter of fact that clamping the carotid did not suspend entirely the circulation in the ear and, in addition, while aseptic precautions were attempted in the slight operation involved, it was not found possible to avoid opportunities for infection. Further experiments along this line will be reported in the future.

SIGNIFICANCE OF THE ACTION OF LIGHT ON BLOOD.

In his work on animals with lymphocytosis, induced by exposure to dry heat, Murphy (23) has shown that mice with a lymphocytosis show a high degree of immunity to certain transplantable cancers, and also a very great increase in their resistance to doses of bovine tubercle bacilli. The resistance to tuberculosis is increased from two to three fold. Amoss, Taylor, and Witherbee (24) also showed that monkeys with greatly reduced lymphocyte counts, as a result of exposure to x-rays, show a much increased susceptibility to an intracerebral inoculation of poliomyelitic virus filtrate. From these results it would seem that any method of inducing a lymphocytosis (heat or far ultra-violet light) would aid in maintaining a high resistance to these diseases at least. It has been suggested, in fact, by Rollier (25) and others that the beneficial effects of heliotherapy in tuberculosis may be due to the action of the actinic rays in increasing the lymphocyte count. Chamberlain, Wickline and others (11, 12, 13, 14, 15), working on the blood count of soldiers in the Philippines, and finding, as a result of the tropical climate, an increased lymphocyte and a decreased polymorphonuclear differential count, took this to indicate a poor physical condition on the part of the men. The chief phagocyte cell of the blood is the polymorphonuclear neutrophile and it is presumably of great importance in the destruction of bacteria in the blood stream. Wickline (12) quotes Cabot as saying "It would appear that the degree of health in persons not organically diseased might perhaps prove to vary directly with the percentage of polymorphonuclear cells in the blood." In Wickline's work (12) the total leucocyte count remained practically constant throughout the nineteen months of examination while the percentage of polymorphonuclears steadily fell, and the percentage of lymphocytes steadily rose. There was therefore an absolute decrease in polymorphonuclears, and this condition may signify an increased susceptibility to bacterial invasions.

If a condition may be obtained in which the absolute number of polymorphonuclears remains practically normal while the absolute number of lymphocytes is greatly increased, as after exposure of rabbits to a bare iron arc, the blood would have normal phagocytic power together with such increased resistance as may depend upon the peculiar activity of the lymphocytes.

CONCLUSIONS.

Direct radiation of rabbits' ears for short periods (one hour) by an iron arc through different filters has shown:

1. The region of far ultra-violet (wave length shorter than 300 $\mu\mu$) has practically no effect on the absolute number of polymorphonuclears, but produces a very marked lymphocytosis, lasting about three weeks.

2. The near ultra-violet (330 to 390 $\mu\mu$) has a marked depressing effect on the lymphocytes, and, to a less degree, on the polymorphonuclears.

3. The region between 450 and 650 $\mu\mu$ has a stimulating action on both lymphocytes and polymorphonuclears, particularly on the former.

4. The wave-lengths longer than 650 $\mu\mu$ (the red and infra-red), produce no effect on the blood beyond the drop in lymphocytes which occurs immediately after exposure to light of any kind.

BIBLIOGRAPHY.

1. Blessing, H. G. Von der Norwegischen Fram-Expedition. Deutsch. med. Wchnschr., 1897, 251.
2. Grober and Sempell, O. Die Blutzusammensetzung bei jahrelanger Entziehung des Sonnenlichtes. Deutsch. Arch. f. klin. Med., 1919, CXXIX, 305.
3. Borissow. Zur Lehre von der Wirkung des Lichts und der Dunkelheit auf den tierischen Organismus. Zeit. f. Diätet. u. physik. Therapie, 1900, V, 337.
4. Graffenberger, L. Versuche über die Veränderungen, welche der Abschluss des Lichts in der chemischen Zusammensetzung des tierischen Organismus und dessen N-Umsatz hervorruft. Archiv. f. d. ges. Physiol., 1893, LIII, 253.
5. Marti, A. Wie wirken die chemischen Hautreize und Belichtung auf die Bildung der roten Blutkörperchen. Verhandl. d. 15. Kongresses f. innere Medizin, 1897, 598.
6. Orum, H. P. T. Ueber die Einwirkung des Lichts auf das Blut. Pflüger's Archiv. f. Physiologie, 1906, CXIV, 1.
7. Polito, G. L'influenza dei Raggi Solari sul Sangue. Gazz. internaz. di Med., 1908, XI, 329.
8. Murphy, J. B., and Sturm, E. Effect of dry heat on the blood count in animals. Jour. of Exp. Med., 1919, XXIX, 1.
9. Aschenheim, E. Der Einfluss der Sonnenstrahlen auf die leukocytäre. Blutzusammensetzung. Zeitschr. f. Kinderheilkunde, 1913, IX, 87.
10. Taylor, H. D. Effect of exposure to the sun on the circulating lymphocytes in man. Jour. Exp. Med., 1919, XXIX, 41.
11. Chamberlain, W. P., and Vedder, E. B. A study of Arneth's nuclear classification of the neutrophiles in healthy and adult males, and the influence thereon of race, complexion and tropical residence. Phil. Jour. Sci. (Sec. B.) 1911, VI, 405.
12. Wickline, W. A. The effects of tropical climate on the white race. Mil. Surgeon, 1908, XXIII, 282.
13. Guerrero, L., and Sevilla, A. Medical survey of the town of Tay Tay. Phil. Jour. Sci. (Sec. B.) 1909, IV, 273.
14. Phalen, J. M. An experiment with orange red underwear. Phil. Jour. Sci. (Sec. B.) 1910, V, 525.

15. Chamberlain, W. P., Bloomberg, and H. D. Kilbourne, E. D. Examinations of stools and blood among the Igorots at Baguio, Philippine Islands. Phil. Jour. Sci. (Sec. B.) 1910, V, 505.
16. Simon, C. E. Clinical diagnosis. Lea and Febiger, Philadelphia, 1918, 31–39.
17. Freer, P. C. The tropical sunlight. Phil. Jour. Sci. (Sec. B.) 1910, V. 1,
18. Miethe, A., and Lehmann, E. Uber das ultraviolette Ende des Sonnenspektrums. Sitzungsber. d. k. Pr. Akad. d. Wissensch., 1909, VIII, 268.
19. Pflüger, A. Die Anwendung der Thermosäule im Ultraviolett und die Energieverteilung in den Funkenspektren der Metalle. Ann. d. Physik., 1904, XIII, 890.
20. Jesionek, A. Lichtbiologie. Vieweg u. Sohn, Braunschweig, 1910, 137–145.
21. Klieneberger, C., and Carl W. Die Blut-Morphologie der Laboratoriums-Tiere. Barth, Leipzig, 1912, 30–31.
22. Howell, W. H., and Holt, E. Two new factors in blood coagulation, heparin and pro-antithrombin. Amer. Jour. Physiol., 1918, XLVII, 328.
23. Murphy, J. B., and Sturm, E. The lymphocytes in natural and induced resistance to transplanted cancer. Jour. Exp. Med., 1919, XXIX, 25.

    Effect of stimulation of the lymphocytes on the rate of growth of spontaneous tumors in mice. Jour. Exp. Med., 1919, XXIX, 31.

    Experiments on the rôle of lymphoid tissue in the resistance to experimental tuberculosis in mice. Jour. Exp. Med., 1919, XXIX, 35.
24. Amoss, H. L., Taylor, H. D., and Witherbee, W. D. Effects of large doses of X-rays on the susceptibility of the monkey to experimental poliomyelitis. Jour. Exp. Med., 1919, XXIX, 115.
25. Roatta, G. B. L-Elioterapia nella pratica medica e nell' educazione. Milan. 1914.

THE NATURE OF SECONDARY VALENCE[1]

BY HOMER W. SMITH

I. PRELIMINARY COMMUNICATION

1. The Concept of Secondary Valence

By secondary valence is meant that force which binds molecules together. Secondary valence is the chemist's name for the force which the physicists call cohesion. Apart from specific cohesion, we know very little about the intimate nature of this force; so little, in fact, that it cannot be said at the present time what part of the atom (or the molecule) is responsible for molecular aggregation.

Physicists who treat the problem of cohesion usually assume that the attraction of one molecule for another is to be attributed to "stray electric fields" surrounding the valence electrons of the atom; they further assume that molecular configuration plays an important part in determining the attraction between two molecules, and that the force acting between two molecules is subject to treatment on the basis of the inverse square, or the inverse fourth power, or some such mathematical function of the distance supposedly separating them. It is within the province of this paper to show that all three of these assumptions are erroneous. Evidence derived from a study of organic compounds will be presented to show that the forces acting between molecules are comparable to those forces in the atom which are responsible for atomic structure, in that they are rhythmic in nature and are consequently not subject to treatment under familiar electromagnetic laws.

Something of the general nature of secondary valence can be deduced from familiar facts. Since all substances may exist in the liquid or solid state, all atomic species must possess some secondary valence. The characteristic intensity of secondary valence associated with any molecular species, however, depends to a great extent upon the arrangement of the atoms in the molecule. This sensitivity to constitutive[1] relations concerns, from a causative point of view, only one or two atoms in the entire molecule. In other words, these variations in molecular activity are attributable solely to variations in the activity of one or two atoms, and they are the result of differences in the mode of chemical combination of these atoms. There are only a few atoms which are capable of varying in their activity in this manner. We shall be concerned in the present study only with oxygen and nitrogen, the two which are undoubtedly the most important.

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[1] This is the first of a series of papers dealing with the nature of secondary valence and the rôle of molecular volume in the phenomena of solution. This investigation has been made in the Department of Physiology, School of Hygiene and Public Health, Johns Hopkins University, and it is submitted in accordance with the requirements for the degree of Doctor of Science in Public Health.

Consider, for example, the compounds valeric acid and water. There certainly is no reason for believing that the molecules of these substances are held together by forces of a dissimilar nature; yet when we come to combine valeric acid with water, some writers go so far as to assume that an entirely new force enters into the intermolecular relations. Others are satisfied to proceed upon the basis that the valeric acid is not combined with the water at all, but that it is in an analogous state to a gas and occupying mythical intermolecular spaces for which there is no experimental justification. Apart from the results of this research, it seems a self-evident deduction that the valeric acid molecules have much the same relation to the solvent as do other molecules of water, and that the same forces are at work between them in identically the same manner.

Yet when we compare the solubility of valeric acid with that of pentane, we see that the introduction of two oxygen atoms has worked a great change in the properties of the latter, for while valeric acid is very soluble in water, pentane is almost insoluble. If now we pass to ethyl valerate, the solubility falls off again to a vanishingly small value. Here then is the problem of constitutive behavior: Two oxygen atoms in the carboxyl group can endow the pentane molecule with properties which render it very soluble in water; but the same oxygens in the ester are essentially as inert as the carbon and hydrogen themselves.

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[1] The term *constitutive* should be carefully distinguished from *configurative*. When atoms are connected by primary valence to make a molecule, certain undefined interactions take place between them which alter their intrinsic nature. Such changes are called *constitutive* since they have to do with chemical *constitution*. The *configuration* of the molecule is a hypothetical feature with which the physical chemist will rarely have to deal.

Under any concept of secondary valence, it must be admitted that the activity of one atom may vary through such wide extremes that this atom can practically determine the molecular behavior.

We have then, in résumé, a force possessed in some measure by all atoms. It may be possessed, however, by certain atoms under particular conditions, in great excess. In such atoms of variable activity, it is clear that the activity depends upon the mode of chemical combination. This is the force which we are now calling secondary valence.

Before we can make any progress in the analysis of the nature of secondary valence, we must introduce into all physical-chemical considerations another factor, namely, molecular volume. When a number of molecules come to positions of equilibrium with regard to electrical forces acting between them, there are obviously two factors brought into play. The first is the respective intensities of the attractions between them; the second is the relative amount of space which they occupy, or their respective molecular volumes.

Molecular volume has received little or no attention in the consideration of intermolecular relations. A study of various physical phenomena has convinced me that molecular volume is a determining factor in practically all the phenomena associated with liquids. By its use we have a means of correlating molecular behavior, and many relations are disclosed which otherwise would go unrecognized.

2. The Concept of Molecular Volume[1]

By molecular volume is meant the volume in cubic centimeters of a gram molecule of a particular substance, determined for certain practical reasons at the normal boiling point. Molecular volumes are additive in nature. That is, values may be obtained through the comparison of a series of compounds for each component atom and these atomic values may then be added together to derive the molecular volume of any desired molecular species. Constitutive variations are very much in evidence; for the volumes of some atoms, especially oxygen and nitrogen, vary by nearly 100 percent, according to the nature of their chemical union. But these constitutive influences are relatively few and constant and the results obtained justify the assumption that LeBas' methods of calculation are fundamentally sound.

We have in some measure been misled by the graphical formulas which we draw upon the blackboard into believing that molecules always have rigid and characteristic configurations. It is almost second nature to think of benzene as being ring-like and of hexane as being chain-like. Such a notion is immediately dispelled by a cursory inspection of molecular volume relations. The very application of the additive principle necessitates the immediate recognition of the fact that every molecule, no matter what its chemical nature, occupies a domain which is the perfect sum (admitting constitutive relations, which of course have to do with internal and not external matters) of the space occupied by the component atoms. This means that there is neither a "hole" inside benzene, nor any "cracks" nor "crevices" in hexane, as there would surely be were either of them composed of rigid spheres of carbon with smaller rigid spheres of hydrogen tacked on at various places. The principle of the perfect sum implies that the six carbons and their attendant hydrogens make up a new domain which has the same *shape* as has each of the components, much as though we had put *several little spheres* together to make *one big sphere.* This rather surprising condition is initially a consequence, as I have said, of the additive nature of molecular volumes, but it is substantiated in an overwhelming manner by the rôle of molecular volume in the phenomena of molecular physics, where there is never any indication that any molecule has any other shape than that of a perfect sphere. That the shape of the molecular domain is not a perfect sphere in the literal sense is obvious from the same argument; for in that case there would be crevices between adjoining domains for which no allowance is made in the calculation of molecular volumes. We must rather look upon this shape as constantly varying between that of a tetrahedron and that of a sphere because of the constant agitation and collisions to which molecules of liquids are subjected. For the purposes of definition it should suffice to call it polyhedral, with the reservation that there can be no "dead" interstices between adjoining domains.

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[1] The molecular volumes used in this paper are either cited from LeBas or calculated according to LeBas' rules. (Gervaise LeBas: The Molecular Volumes of Liquid Chemical Compounds, Longmans, Green and Co., New York, 1915). A brief of LeBas' methods has been given in a previous communication. Jour. Phys. Chem., **24**, 539 (1920).

It is difficult to conceive of any atomic theory so mercurial as to explain satisfactorily the facts disclosed by a study of molecular volumes.

The atom is supposed to be a very porous affair, about as porous as the solar system. All physical experience shows us, however, that the space between the particles of electricity which compose the atom is so filled or constrained by lines of force that no two atoms can occupy the same space at the same time, or even overlap beyond certain assumed limits. Atoms thus held together by electrical forces constitute the molecule in which the atomic arrangement accounts for the chemical identity, which is never lost. We have just seen that the space relations of molecules are vastly different from what we would expect from the permanence of this atomic arrangement. The molecular volume at the boiling point is about three times what it is at absolute zero. This increase in volume is sometimes attributed to increased molecular motion. The validity of this assumption is open to question, but even if we do assume that any part of the *molecular volume* in the liquid state (which, for the purposes of this paper, is synonymous with *molecular domain*) is due to molecular motion, this fraction must be statistically identical under like conditions for various molecular species. It thus becomes a universal factor which may be omitted from immediate consideration. To all intents and purposes the molecular domain is *completely filled* by the molecule. The boundary of the domain is purely an imaginary surface within which no other molecule can transgress. As to what the actual dimensions of the molecule may be, and as to the proportions of this actual volume and the volume of the apparent domain, we need not be concerned at the present time. The essential idea to be gained from an analysis of molecular volume relations is that *every molecule behaves as though it completely fills a definite space which has three-dimensional symmetry.*

Secondary valence, as has been said, is the force which binds molecules together. We must picture this union as being quite labile, for above the melting point there must be considerable molecular motion, crudely analogous to the motion of shot in a well-filled bucket which is being vigorously agitated.[1] *As a statistical result of this motion* and in keeping with the foregoing concept of molecular domains, we are entitled to think of secondary valence as acting as though it were either uniformly distributed over the surface of the molecular domain, or localized at its center. When molecular motion is restricted, as it must be at the interfaces of liquids, the molecule behaves as though its secondary valence were localized at one or more points. (It is noteworthy that here molecular volume relations fail, and the molecule assumes a definite configuration or shape.) But there is no evidence to indicate that such a localization ever takes place when the molecule is completely and intimately surrounded by other molecules. In those molecules where two or more atoms actually contribute to the total secondary valence, the net result is apparently the same as though the entire secondary valence were to be attributed to one source.

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[1] Some molecular motion in liquids is a necessary assumption to account for vapor tension, solute diffusion and the absence of a structure. But a satisfactory explanation of all these could be based, it seems, just as well upon rotational motion as upon translational motion. If this motion is assumed to be largely rotational, it is evident that it would contribute but little to the molecular volume.

3. Partition Coefficients

The phenomena of solution, from a practical point of view, are the most important phases of molecular behavior with which the physical chemist has to deal. Solubility relations are likewise the most susceptible to constitutive influences. On this basis, no better field could be chosen for the study of the nature of the forces acting between molecules. But a study of absolute solubilities is impractical because most of the lower members of chemically homologous series are miscible with water in all proportions, and hence they are excluded from direct consideration.

The problem may be approached, however, through a study of partition coefficients; for when a solute distributes itself between two immiscible liquids the amount in each of the liquids is an index of the relative solubility of the solute in the respective liquids. By determining the partition coefficients of a large number of compounds between any two immiscible liquids, such as water and xylene, it is possible to obtain an idea of the nature of the electrical forces involved in solution.

This method, though very simple and practical, presents several theoretical difficulties. For practical reasons we must work with titratable substances, using water as one solvent. These substances will undergo ionization in water and many of them will also be associated in the organic solvent. Since there is relatively no dissociation in organic solvents and no association in water, the ionized and associated fractions are removed from the system, so to speak, for neither can pass, as such, to the other liquid. In consequence, as smaller total concentrations are used, both of these processes tend to lower the partition coefficient. In order to obtain a figure which is constant and characteristic of the solute, it is necessary to make a correction for these disturbing factors. Moreover, the second solvent may dissolve in the water and the water may dissolve in the second solvent, thus disturbing the normal distribution and the dissociation and association constants. In some cases a fifth disturbing factor is introduced in the formation of hydrates. After prolonged but vain attempts to establish a method of correction based upon stoichiometric equations and applicable to all cases, it was found necessary to adopt an empirical method of correction. This method will be discussed in more detail in a subsequent communication. The validity of the method can be established apart from the general results which it yields by the following facts:

(1) The partition coefficients of chemically related compounds between water and one solvent bear a constant ratio to the partition coefficients between water and a second solvent.

(2) The partition coefficients of two compounds bear a constant relation to each other regardless of the nature of the solute, the total concentration, or the nature of the second solvent.

(3) The partition coefficient of a compound may be derived independently of the relative amounts of water and second solvent used.

Conditions one and two are subject to certain well-defined exceptions which can only be treated in the discussion of the experimental work. From a consideration of the distribution law, it will be seen that these conditions are just what would be expected if all disturbing factors were eliminated; therefore they indicate that the method of correction is valid and that any relations disclosed by its use are, from a physical-chemical point of view, not open to question.

The partition coefficients of all the simpler acids and amines have been determined between water and xylene and water and chloroform, and in a few cases between water and other solvents. The relations between these partition coefficients have been analyzed in terms of molecular volume. The principal facts disclosed by this analysis are as follows:

In any series of compounds having the same intrinsic intensity of secondary valence, the partition coefficient is a simple logarithmic function of the molecular volume.

Secondary valence is rhythmic in nature; that is, it varies in different molecular species by definite and related amounts. These differences in secondary valence are expressed in the partition coefficient by increases or decreases of constant arithmetic value. These variations in secondary valence are attributable to variations in the state of one or two particular atoms in the molecule and they have their origin in the mode of chemical combination. In all of the compounds studied, these rhythmic variations appear to be identical in nature; hence it is assumed that secondary valence is to be attributed to some part of the atom which is common and identical in its nature in all atomic species.

From the foregoing it can be seen that this atomic mechanism must function in a rhythmic fashion and that its activity is determined primarily by the mode of chemical combination.

The secondary valence associated with a given molecular species depends, however, not only upon its chemical nature, but also upon the nature of its environment. This fact, coupled with the fact that molecular volume is a determining factor in the relations of the solute to the solvent, shows that there are equilibrated intermolecular forces acting between the molecules of the solvent and the molecules of the solute, and that these intermolecular forces arise and are compensated in the underlying mechanism in those atoms of variable activity which are responsible for the characteristic secondary valence of the molecule.

Thus in such a compound as valeric acid, the mode of chemical combination of the (hydroxylic) oxygen determines the characteristic secondary valence of the molecule. When such a molecule is placed in a dissimilar environment, such as water, a compensation between the external and internal forces must take place; this compensation is effected like all changes in the secondary valence of the molecule, by a change in the oxygen atom. Since this change involves some readjustment of the valence electrons, we can see in these facts a possible mechanistic cause for dissociation.

All these changes in secondary valence are rhythmic in nature presumably because they have their origin in some rhythmic mechanism in the atom analogous to the stable electronic orbits which have been postulated to account for Planck's quantum in energy radiation.

So far as I am aware, this is the first direct experimental demonstration of a quantum, or rhythmic variation, in the fundamental forces. The results are of interest not only in their relation to the nature of solution, but because they have a bearing upon the structure of the atom.

A detailed consideration of partition coefficients will be given in the next communication.

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THE NATURE OF SECONDARY VALENCE[1]

BY HOMER W. SMITH

PART II. PARTITION COEFFICIENTS

§1. Introduction

When an aqueous solution of any substance is shaken with an immiscible liquid some of the solute will pass from the water to the other liquid. The ratio of the concentration of the solute in the second liquid to the concentration in the water, after equilibrium has been attained, is called the distribution or partition coefficient. Under ideal conditions, the amount of solute passing into the second liquid would be proportionate to the concentration in the water, and the partition coefficient would be constant and independent of the total concentration; that is, we might write $C_2/C_1 = k$. This simple state of affairs is rarely met with, however, for practically all the substances with which we are concerned are dissociated in water, and many of them are associated in organic solvents. Since electrolytic ions do not exist in a non-dissociating solvent, such as xylene, and since associated molecules do not exist at great dilution in a dissociating solvent, such as water, both the dissociated and associated fractions of the solute in the two respective layers are removed from the system, so to speak, for neither can pass, as such, to the other layer. Hence, at a given total concentration, dissociation tends to lower the partition coefficient and association to raise it.

Any attempt to compare the partition coefficients of various substances would obviously be futile until both of these disturbing factors have been eliminated. Dissociation, which proceeds according to well known laws, can be corrected for on the assumption that the concentration in the second phase is proportionate to the undissociated fraction in the water. From the dissociation constant we can calculate the percentage dissociation at a dilution equivalent to the concentration in the water when equilibrium has been reached, and the partition coefficient then becomes: $C_2/C_1(1 - a) = k$. But there are only a few instances where this correction for dissociation suffices to hold the partition ratio constant over a large range of total concentrations, for most compounds, especially among the organic acids, are associated in organic solvents.

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[1] From the Department of Physiology, School of Hygiene and Public Health, Johns Hopkins University. The author is indebted to Mrs. R. A. Spaeth for generous assistance in the preparation and revision of the manuscript.

The mechanism involved in this association is comparable to that of dissociation, if we but think of the associated molecules in the organic solvent as similar to the undissociated molecules in water, and the single molecules in the organic solvent as similar to electrolytic ions. In concentrated solutions a larger fraction of the solute will be associated than in dilute solutions, and for intermediate concentrations all progressive degrees of decomposition of the associated fractions will be possible. But the analogy is imperfect, in that the maximum degree of association is unlimited; that is, the solute may be associated into aggregations of two, three, four, etc., molecules. Such aggregations as exist, however, would be expected to decompose at a rate constant and characteristic for each molecular species, comparable in a sense to the rate at which the single molecules decompose into electrolytic ions in water. This "decomposition constant" might be determined by studying both concentrated and dilute solutions, but there are reasons to believe that even this would not suffice to untangle the web. Water will dissolve in the second solvent, and thus upset the association constant, and the second solvent will dissolve in the water, and upset the dissociation constant. Furthermore, in some cases the partition coefficient increases with decreasing total concentration. This cannot be accounted for on the basis of either association in the second liquid, or dissociation in the water. It may be due to hydration. So complex do these disturbing factors become that it seems almost futile to attempt to correct for them on a stoichiometric basis. For our purposes it is necessary to have values which represent the true tendency of the various species to pass from the water to the other solvent and any variations introduced by these disturbing factors would materially affect the interpretations to be based upon them. We have no way of knowing whether we have eliminated the disturbing factors or not, except as we anticipate in some measure the results which we may reasonably hope to obtain.

Before beginning a search for a suitable method of correction, several conditions were set down which any correction, to be satisfactory, would be expected to meet. If we compare the partition coefficients of a series of allied compounds, determined at the same total concentrations, into two solvents, such as chloroform and xylene, we see that the partition coefficients into the one solvent bear *approximately a constant ratio* to the partition coefficients into the other. This ratio is maintained fairly well so long as the compounds investigated have a similar dissociation constant; but when we include a compound which departs from the average in this respect, the ratio is materially altered. It seems a reasonable assumption that, if properly corrected for dissociation and association (if this latter occurs), this ratio would prove to be a constant. If we designate the *properly corrected* partition coefficient by $\mathfrak{P}$, we might then write

$$\frac{\mathfrak{P}_{\text{Chloroform}}}{\mathfrak{P}_{\text{Xylene}}} = \text{Constant}.$$

This simple relation should apply at least to nearly related substances, if we may judge from our experience with uncorrected partition coefficients. Furthermore, the ratio between the partition coefficients of any two compounds should be maintained regardless of the concentration at which the partition coefficients are determined, the nature of the solutes, and the nature of the second solvent. Since we are looking for certain relations among homologous compounds, we may in fairness anticipate them and require that our method of correction yield results which are subject to a reasonable interpretation. It would follow from the previous conditions that this interpretation would be essentially independent of the nature of the second solvent.

It would hardly be worth while to fill up several pages with test cases showing how methods of correction based upon dissociation constants and association factors fail in one or more respects to fulfill the above requirements. It is more to the point to discuss briefly the only method I have found so far which does appear to be satisfactory. It is unfortunately empirical and it cannot be said to be founded upon any physical-chemical experience whatsoever. The sole excuse for its use lies in the results obtained by it, and on these results alone can one base an opinion as to its validity.

We will consider the corrected partition coefficient, $\mathfrak{P}$, to be the ratio of the concentration in the second solvent to the concentration in the water (C_2/C_1) when the *concentration in the second solvent has some arbitrary and unitary value.* That is, $\mathfrak{P} = C_2/C_1$ when C_2 is 100 millimols, 10 millimols, 1 millimol, etc., per liter. This is similar to the method first advocated by Nernst; *i. e.*, deriving a constant ratio through the use of an exponential equation: $\frac{C_2}{C_1^y} = k$, where y is an uncharacterized exponent. The method differs from Nernst's in that no mathematical manipulation is involved, for in no case is y constant over a large range of total concentrations, and consequently the equation can never be ideally fulfilled. But we can determine the partition coefficient at suitable total concentrations so that we will actually pass, or approach very close to, a given concentration in the second solvent, and we thus reduce the possibility of introducing error through interpolation to a minimum.

A few cases have been cited from the literature to illustrate the application of this method of correction. If we compare the partition coefficients of several substances between water and several solvents, all of which solvents are relatively insoluble in water, we find that the ratio between the partition coefficients into one solvent and the partition coefficients into another solvent is a constant, and that it is independent of the concentration chosen for C_2.

The data upon which the following interpolations have been based are given in full in Section I of the tables given in the experimental part of this paper. Below I have given only the concentration in the second solvent to which interpolation is made, the corrected partition coefficients, and the ratios between these partition coefficients.

TABLE I

	C_2 (m L)	$\mathfrak{p}$ CCl_4	$\mathfrak{p}$ Toluene	$\frac{\mathfrak{p}\ \text{Tol.}}{\mathfrak{p}\ CCl_4}$	$\mathfrak{p}$ Benzene	$\frac{\mathfrak{p}\ \text{Benzene}}{\mathfrak{p}\ \text{Toluene}}$
Acetic acid	1000.0	0.10	0.12	1.20	—	—
Chloroacetic acid	1000.0	0.06	0.07	1.17	—	—
Phenol	1000.0	2.45	2.75?	1.12	4.3	1.56
Trimethylamine	1000.0	—	0.43	—	0.50	1.16
Pyridine	100.0	—	2.06	—	2.60	1.34
Chloroacetic acid	100.0	—	0.028	—	0.036	1.30
Butyric acid	1.0	0.115	0.16	1.39	0.215	1.26

It will be noticed that here we have a weak acid, a moderately strong acid, an alcohol, a weak base, and a ketone. Some of these we know to be associated in organic solvents; most of them we know are dissociated in water; nevertheless the ratios of the partition coefficients between water and carbon tetrachloride, and water and the other solvents are fairly constant. It is quite impossible to get this result with any other method of correction, especially with those compounds which show association in the organic liquids.

If, now, one of the solvents under comparison is soluble in water, the ratios vary with the different arbitrary values for C_2 which are chosen for extrapolation. Thus chloroform is much more soluble in water than is benzene or toluene. The ratio of the more soluble solvent over the less soluble one increases as lower values are chosen for C_2.

TABLE II

	C_2 (m L)	$\mathfrak{p}$ CHCl	$\mathfrak{p}$ Benzene	$\frac{\mathfrak{p}\ CHCl_3}{\mathfrak{p}\ \text{Benzene}}$	$\mathfrak{p}$ Toluene	$\frac{\mathfrak{p}\ CHCl_3}{\mathfrak{p}\ \text{Tol.}}$
Acetic acid	1000.0	0.20	—	—	0.12	1.67
Phenol	1000.0	5.50	4.3	1.28	—	—
Chloroacetic acid	100.0	0.062	0.0365	1.70	0.028	2.22
Acetic acid	100.0	0.10	0.054	1.85	—	—
Butyric acid	1.0	0.385	0.215	1.79	0.16	2.40

If the two solvents are equally soluble in water, this divergence disappears, as can be seen from the comparison of chloroform and bromoform.

TABLE III

	C_2 (*m* L)	$\mathfrak{p}$ $CHCl_3$	$\mathfrak{p}$ $CHBr_3$	$\frac{\mathfrak{p}\ CHCl_3}{\mathfrak{p}\ CHBr_3}$
Acetic acid	1000.0	0.20	0.148	1.35
Phenol	1000.0	5.50	4.30	1.28
Chloroacetic acid	1000.0	0.125	0.094	1.33
Ammonium	100.0	0.038	0.029	1.31
Picric acid	100.0	2.20	1.60	1.37

Though the evidence which has been given thus far is not very comprehensive, it is sufficient to show that so long as we use the same arbitrary value for C_2 for interpolation, the partition coefficients of various species so derived into any one solvent are subject to comparison; for since the partition coefficients of several compounds into two or more solvents bear a constant ratio to each other, all irregular influences arising in the nature of the second solvent are eliminated.

The values of the partition coefficient for any two compounds so obtained maintain a constant ratio to each other regardless of the nature of the second solvent, the total concentration, or the chemical nature of the solute. The truth of this last condition can be seen in Table IV.

TABLE IV

	C_2 (*m* L)	$\mathfrak{p}$ Acetic acid	$\mathfrak{p}$ Chloroacetic acid	$\frac{\mathfrak{p}\ \text{Acetic acid}}{\mathfrak{p}\ \text{Chloroacetic acid}}$
Chloroform	1000.0	0.20	0.125	1.60
Bromoform	1000.0	0.148	0.094	1.57
Toluene	1000.0	0.12	0.07	1.71
Carbon tetrachloride	1000.0	0.10	0.06	1.66
Chloroform	100.0	0.10	0.062	1.61
Benzene	100.0	0.054	0.0365	1.48
Chloroform	1.0	0.0725	0.0438	1.65
Xylene	1.0	0.030	0.0175	1.71

The partition coefficient is independent of the relative amounts of water and second solvent used.

Table V

Phenylacetic Acid into Xylene

	C_2	$\mathfrak{P}$
1 vol. xylene to 1 vol. water	1*m*/L	0.510 × 1 = 0.510
1 vol. xylene to 2 vols. water	0.5*m*/L	0.253 × 2 = 0.506
2 vols. xylene to 1 vol. water	2*m*/L	1.02 ÷ 2 = 0.510

We have now obtained fixed values for these partition coefficients where otherwise they would move indefinitely through a wide range because of at least five disturbing factors. The relative concentrations of any solute in the two solvents are indicative of the relative solubilities of the solute in those solvents. And since the concentration in the second solvent is always the same, the concentration in the water becomes a measure of the tendency of that solute to leave the aqueous environment and pass into the other environment, or a measure of the molecular fugacity. Further evidence will be brought out in the discussion to substantiate the method of correction, for by it are revealed many relations which have never been revealed by any other method of correction.

But it must be kept in mind that comparisons are valid only between values of $\mathfrak{P}$ derived from the same arbitrary concentration in the second solvent (C_2). For the purposes of this paper, the optional value of C_2 chosen for interpolation is 1.0 millimol per liter, for nearly all acids and bases are sufficiently soluble in water to permit our passing this concentration experimentally. A very short interpolation between values for C greater than 1 *m*/L and less than 1 *m*/L is all that is necessary to obtain the corrected partition coefficient $\mathfrak{P}$. In only a few cases (and the majority of these are cited from the literature) has this interpolation been made from values entirely above, or entirely below, this arbitrary concentration.

§2. Experimental

Aqueous solutions of known titer were shaken for three to five minutes with equal volumes of the second solvent. The mixture during shaking was contained in either a separatory funnel or a stoppered Erlenmeyer flask. Previous to shaking, the containing flasks and the solutions were brought to 25° ± 0.5, by immersion in a water bath, and after shaking the mixture was allowed to separate in the water bath. After separation, portions of the aqueous layer were titrated, and the concentration in the second solvent was determined by difference. The volatile nature of most of the materials studied and the extent of the work made it impractical to determine the concentrations in the second solvent directly. All titrations were made with standard hydrochloric acid and sodium hydroxide solutions, using phenolsulphonphthalein and methyl orange.

The bases were, for the most part, from freshly opened bottles of Kahlbaum's C. P. Reagents. For these, and for some of the acids, I am greatly indebted to Dr. Carl Voegtlin, whose co-operation in this matter has gone far to make this work possible. I am also indebted to Dr. E. K. Marshall, Jr., for some of the bases. The remaining preparations were secured from the Eastman Kodak Company. Inasmuch as this research was intended as a preliminary survey of the field, no attempt was made to obtain a degree of accuracy commensurate with physical research. None of the liquid chemical compounds was repurified, though practically all the crystalline compounds were recrystallized. The greatest difficulty in securing suitable checks lies in the extracting solvent. A slight contamination here of the proper sort will prove very disastrous. The xylene and chloroform were carefully repurified by shaking with strong alkali and then with 5 percent sulphuric acid, and by redistilling over a mixture of calcium oxide and calcium chloride. The other solvents were similarly repurified. The method offers little experimental difficulty, but a few sources of systematic error were found which may be worth mentioning. Solutions of both amines and acids will decrease greatly in strength if violently shaken for a few moments, due evidently to increased surface adsorption. This adsorbed material diffuses very slowly into the solution again, and to guard against this effect, a sufficient interval of time should be allowed to elapse between sampling. The effect is especially pronounced immediately after making a solution of a liquid chemical compound, and after emulsification with an immiscible solvent. Five to ten minutes were always allowed after shaking to let the solution regain "diffuse" equilibrium. The amines, being very volatile, are easily lost from their aqueous solutions and the water layer should not be run out of a separatory funnel before titration. Samples can be taken direct from the funnel by blowing a small bubble of air through the pipette to expel the other solvent. Phenolsulphonphthalein gives a clean end-point with nearly all the amines (except the anilines of course), but it is not so satisfactory with the weaker acids. Where the partition coefficient is very small, an obscure end-point makes the determination very difficult. It is as satisfactory, however, as any other indicator for the purpose, and has the advantage that one can tell with it just what carbon dioxide is doing. By sucking through the pipette before sampling to clear out any carbon dioxide from the breath, difficulties from this source were eliminated.

In the following tables, the first section comprises material quoted from the literature, and the second section, the original contributions. The column headings need no explanation beyond the statement that unless otherwise indicated, all concentrations are expressed in *millimols per liter.*

SECTION I

	C_1	C_2	C_2/C_1	𝔭	Reference[1]
BENZENE:					
Acetic acid, 25°	m/10 cc	m/10 cc			
	32.98	2.559	0.0776		
	25.867	1.580	0.061		
	13.821	0.554	0.040	1m/10 cc	
	5.793	0.159	0.027	0.054	H. and F.
Chloroacetic acid, 25°	m/10 cc	m/10 cc			
	71.856	3.177	0.0442		
	63.916	2.533	0.0396		
	21.191	0.729	0.0344	1m/10 cc	
	16.049	0.522	0.0325	0.0365	H. and F.
Phenol, 25°	m/cc	m/cc			
	0.3660	2.978	8.13		
	0.2578	1.254	4.87		
	0.1013	0.279	2.75	1m/cc	
	0.0272	0.062	2.29	4.3	R. and W.
Pyridine, 25°	m/0.05 cc	m/0.05 cc			
	0.000380	0.00110	2.9		
	0.000208	0.000546	2.62	1m/10 cc	
	0.000112	0.000274	2.50	2.6	H. and S.
Trimethylene, 25°	m/10 cc	m/10 cc			
	2.474	1.237	0.500		
	1.377	0.670	0.486	1m/10 cc	
	0.584	0.295	0.505	0.5	H. and F.

SECTION I (*Continued*)

	C_1	C_2	C_2/C_1	$\mathfrak{p}$	Reference[1]
BROMOFORM:					
Acetic acid, 25°	m/cc	m/cc			
	10.2	2.062	0.202		
	8.74	1.430	0.164	1m/cc	
	5.37	0.593	0.110	0.148	H. and L.
Ammonium, 25°	m/cc	m/cc			
	6.30	0.235	0.0373		
	4.18	0.131	0.0312	1m/10 cc	
	2.59	0.070	0.0271	0.029	H. and L.
Chloroacetic acid, 25°	m/cc	m/cc			
	9.69	1.20	0.124		
	9.61	0.813	0.084	1m/cc	
	8.85	0.649	0.073	0.094	H. and L.
Phenol, 25°	m/cc	m/cc			
	0.364	2.70	7.42		
	0.304	1.80	5.89	1m/cc	
	0.109	0.322	2.96	4.3	H. and L.
Picric acid, 25°	m/10 cc	m/10 cc			
	0.674	1.140	1.69		
	0.575	0.871	1.51	1m/10 cc	
CARBON TETRACHLORIDE:	0.475	0.655	1.38	1.6	H. and L.
Acetic acid, 25°	m/cc	m/cc			
	9.69	1.07	0.110		
	7.98	0.725	0.091	1m/cc	
	4.87	0.292	0.060	0.10	H. and L.

SECTION I (*Continued*)

	C_1	C_2	C_2/C_1	𝔭	Reference[1]
Chloroacetic acid, 25°	m/cc	m/cc			
	11.3	0.551	0.0486		
	11.1	0.443	0.0398		
	10.7	0.362	0.0339		
	10.3	0.234	0.0229	1m/cc	
	9.6	0.159	0.0166	0.06	H. and L.
Phenol, 25°	m/cc	m/cc			
	0.489	1.47	3.01		
	0.213	0.141	0.665	1m/cc	
	0.140	0.072	0.514	2.45	H. and L.
CHLOROFORM.					
Acetic acid, 25°	m/cc	m/cc			
	2.056	0.349	0.169	1m/cc	
	1.527	0.207	0.136	0.20	
	1.188	0.135	0.114	1m/10 cc	
				0.10	R. and W.
20°	0.2696	0.01222	0.0453		
	0.1946	0.007586	0.0390		
	0.1269	0.00406	0.0320	1m/1000 cc	
	0.06445	0.00174	0.0270	0.025	D. and G.
Ammonium, 20°	gm/L	gm/L			
	7.861	0.2991	0.038		
	6.393	0.2439	0.038	1m/10 cc	
	5.160	0.1958	0.038	0.038	D. and C.

SECTION I (*Continued*)

	C_1	C_2	C_2/C_1	𝔭	Reference[1]
Chloroacetic acid, 25°	m/cc	m/cc			
	7.70	0.840	0.109	1m/cc	
	7.23	0.707	0.0979	0.125	
	6.96	0.603	0.0866		
	3.65	0.253	0.0694	1m/10 cc	
	1.05	0.061	0.0590	0.062	H. and L.
Phenol, 25°	m/cc	m/cc			
	0.330	3.360	10.2		
	0.247	1.850	7.47	1m/cc	
	0.163	0.761	4.68	5.5	H. and L.
Picric acid, 25°	m/10 cc	m/10 cc			
	0.561	1.41	2.52		
	0.488	1.09	2.23	1m/10 cc	
	0.329	0.547	1.66	2.2	H. and L.
TOLUENE:					
Acetic acid, 25°	m/cc	m/cc			
	5.401	0.4477	0.0828		
	2.063	0.0864	0.0418		
	1.117	0.0343	0.0293	1m/1000 cc	
	0.334	0.0060	0.0181	0.010	H. and F.
25°	m/10 cc	m/10 cc			
	95.10	12.69	0.136	1m/cc	
	62.61	5.75	0.0919	0.12	H. and F.

SECTION I (*Continued*)

	C_1	C_2	C_2/C_1	$\mathfrak{p}$	Reference[1]
Chloroacetic acid, 25°	m/10 cc	m/10 cc			
	92.04	5.434	0.059	1 m/cc	
	80.68	3.358	0.0416	0.07	
	54.50	1.699	0.0312	1 m/10 cc	
	33.46	0.926	0.0277	0.028	H. and F.
Phenol, 25°	m/10 cc	m/10 cc			
	4.75	14.96	3.15	1 m/cc	
	2.20	4.66	2.12	2.75	H. and V.
Pyridine, 25°	m/cc	m/cc			
	0.0517	0.1129	2.18		
	0.0132	0.0275	2.08		
	0.0033	0.0066	2.00	1 m/10 cc	
	0.0007	0.0010	1.44	2.06	H. and V.
Trimethylamine, 25°	m/cc	m/cc			
	0.0819	0.0363	0.443		
	0.0408	0.0177	0.433	1 m/10 cc	
	0.0206	0.0087	0.422	0.43	H. and V.

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[1] H. and F. Herz und Fischer: Ber. deutsch. chem. Ges., **37**, 4746 (1904); **38**, 1138 (1905). R. and W. Rothmund und Wilsmore: Zeit. phys. Chem., **40**, 611 (1902). H. and S. Hantzsch und Sebaldt: Zeit. phys. Chem., **30**, 258 (1899). H. and L. Herz und Lewy: Zeit. Elektrochem., **1905**, 818; Sitzungsber. Schl. Ges. vaterländische Kultur; Naturwissenschäftl. Sektion, **1906**, 1. D. and C. Dawson and McCrae: Jour. Chem. Soc., **79**, 493 (1901). H. and V. Hantzsch und Vogt: Zeit. phys. Chem., 38, 705 (1901). See also W. Herz. Sammlung chemischer und Chemisch technischer Vorträge, **15**, 1 (1910–11).

SECTION I (*Continued*)

	C_1 / m cc	C_1/C_2	Average value $C_1/C_2(1-a)$	𝔭 (C_2 1m/1000 cc)
ETHER:[2]				
Oxalic acid	0.1339	17.77	—	
	0.0887	21.66	—	
	0.0198	42.54	—	
	0.0103	61.38	—	
	0.0054	100.00	8.64	0.03
Malonic acid	0.1478	10.94	—	—
	0.1121	11.07	—	—
	0.0862	11.28	—	—
	0.0331	12.22	9.83	0.077
Succinic acid	0.0217	7.81	—	—
	0.0121	7.95	—	—
	0.0059	8.39	—	—
	0.0039	8.42	—	—
	0.0023	8.79	7.45	0.123
Glutaric acid	0.0280	3.72		—
	0.0085	3.84	—	—
	0.0072	3.91	—	—
	0.0063	3.92	—	—
	0.0056	3.93	3.58	0.253

SECTION I (*Continued*)

	C_1 *m*/cc	C_1/C_2	Average value $C_1/C_2(1 - a)$	$\mathfrak{p}$ (C_2 1*m*/1000 cc)	
Pimelic acid	0.0098	0.7095	—	—	
	0.00702	0.7170	—	—	
	0.00480	0.7195	—	—	
	0.00284	0.7480	—	—	
	0.00179	0.7075	0.664	1.39	
Suberic acid	0.00986	0.215	—	—	
	0.00544	0.228	—	—	
	0.00175	0.246	—	—	
	0.00084	0.258	—	—	
	0.00049	0.274	0.212	3.45	
Azelaic acid	0.00064	0.0782	—	—	
	0.00058	0.0800	—	—	
	0.00051	0.0810	—	—	
	0.00046	0.0823	—	—	
	0.00033	0.0868	0.0637	9.5	
Maleic acid	0.0253	13.27	—	—	
	0.0196	14.40	—	—	
	0.0143	16.06	—	—	
	0.0100	17.78	—	—	
	0.0054	23.70	6.71	0.0668	

Chandler Jour. Am. Chem. Soc., **30,** 696 (1908). Only a part of C.'s work is quoted in the above tables. The figures given in the third column for $C_1/C_2(1 - a)$ are averages derived from a large number of concentrations. Note that in column 4 $\mathfrak{p} = C_2/C_1$, not C_1/C_2 as given by C

SECTION II

The Partition Coefficients of the Acids between Water and Xylene, 25°

	C_1	C_2	C_2/C_1	𝔓
Acetylsalicylic acid	17.30	7.25	0.419	—
	14.62	5.38	0.368	—
	7.15	2.00	0.280	—
	3.85	0.875	0.227	—
	3.28	0.719	0.219	0.222
iso-Amylacetic acid	0.85	7.95	9.37	—
	0.375	3.625	9.67	—
	0.212	2.170	10.25	—
	0.0792	0.8580	10.85	10.70
Anisic acid	0.682	1.50	2.20	—
	0.467	0.700	1.50	—
	0.381	0.494	1.30	—
	0.243	0.195	0.802	1.80
Benzilic acid	1.252	0.975	0.788	—
	1.029	0.643	0.625	—
	0.757	0.363	0.479	—
	0.448	0.133	0.300	0.800
Benzoic acid	4.19	17.4	4.15	—
	1.568	2.90	1.85	—
	0.842	0.865	1.03	—
	0.794	0.819	1.03	—
	0.737	0.678	0.92	1.10
Bromoacetic acid	37.40	1.15	0.0325	—
	26.70	0.90	0.0337	—
	23.30	0.70	0.0300	0.033
α-Bromopropionic acid	13.10	1.40	0.107	—
	10.50	1.00	0.095	—
	8.40	0.70	0.0835	—
	6.20	0.44	0.071	0.095
Bromosuccinic acid	61.70	1.85	0.03	—
	25.90	0.555	0.0214	0.0252
n-Butyric acid	8.20	1.30	0.159	—
	6.475	1.025	0.158	—
	4.500	0.700	0.156	0.158
sec.-Butyric acid	18.35	3.50	0.19	—
	9.575	1.625	0.17	—
	3.65	0.575	0.157	0.160

SECTION II (*Continued*)

	C_1	C_2	C_2/C_1	$\mathfrak{p}$
Camphoric acid	19.166	0.834	0.0436	—
	9.722	0.278	0.0286	—
	3.922	0.0776	0.0198	0.048
n-Caproic acid	2.80	17.7	6.32	—
	0.95	2.95	3.10	—
	0.75	2.15	2.86	—
	0.575	1.50	2.61	—
	0.3375	0.7375	2.18	—
	0.2625	0.5375	2.04	2.37
iso-Caproic acid	1.85	5.6	3.02	—
	1.325	3.375	2.54	—
	0.8125	1.6375	2.02	—
	0.4625	0.7500	1.62	1.75
o-Chlorobenzoic acid	4.55	7.45	1.63	—
	2.30	2.20	0.956	—
	1.75	1.325	0.757	—
	1.55	0.975	0.630	—
	1.19	0.600	0.505	0.635
Cinnamic acid	0.295	2.215	7.45	—
	0.225	1.025	4.55	—
	0.160	0.465	2.90	4.50
α-Crotonic acid	13.9	2.5	0.180	—
	6.3	0.80	0.127	—
	4.75	0.55	0.115	0.135
α-β-Dibromopropionic acid	7.35	1.45	0.197	—
	7.20	1.30	0.181	—
	5.20	0.75	0.144	—
	3.70	0.50	0.135	0.168
2,4-Dinitrobenzoic acid	10.95	1.10	0.100	—
	8.60	0.70	0.0815	—
	7.85	0.55	0.070	—
	6.78	0.40	0.059	0.10
3,5-Dinitrobenzoic acid	4.05	0.50	1.11	—
	3.15	2.95	0.936	—
	2.40	2.10	0.875	—
	1.53	1.11	0.720	0.70
Gentisinic acid	37.50	0.95	0.024	—
	30.50	0.61	0.020	—
	23.55	0.40	0.017	0.0242

SECTION II (*Continued*)

	C_1	C_2	C_2/C_1	$\mathfrak{p}$
o-Iodobenzoic acid	1.10	2.20	2.00	—
	0.60	0.55	0.916	—
	0.325	0.175	0.538	1.15
β-Iodopropionic acid	13.75	4.46	0.325	—
	7.25	1.75	0.242	—
	6.60	1.44	0.218	—
	3.90	0.745	0.191	—
	2.97	0.525	0.1765	0.208
Hydrocinnamic acid	1.15	5.00	4.35	—
	0.85	3.40	4.00	—
	0.575	1.775	3.09	—
	0.375	0.975	2.60	2.75
Laevulinic acid	51.7	1.40	0.0271	—
	26.0	0.50	0.0192	—
	35.0	0.80	0.0228	0.0242
-Nitrobenzoic acid	10.30	0.90	0.0874	—
	7.60	0.70	0.0920	—
	5.70	0.55	0.0965	—
	3.925	0.425	0.1080	0.085
m-Nitrobenzoic acid (1st recrystallization)	2.85	3.25	1.14	—
	2.30	2.30	1.00	—
	1.85	1.60	0.865	—
	1.325	0.95	0.718	0.73
m-Nitrobenzoic acid (2nd recrystallization)	3.00	3.35	1.11	—
	2.20	2.10	0.955	—
	1.60	1.375	0.860	—
	1.20	0.850	0.708	0.74
Nitrobenzoic acid	1.10	0.950	0.864	—
	0.962	0.7375	0.767	—
	0.725	0.4625	0.638	—
	0.681	0.4530	0.665	—
	0.3825	0.1750	0.458	0.90
Phenylacetic acid	5.00	5.00	1.00	—
	3.50	2.473	0.708	—
	2.60	1.5375	0.592	—
	1.60	0.7750	0.484	—
	1.26	0.5750	0.456	0.51

SECTION II *(Continued)*

	C_1	C_2	C_2/C_1	$\mathfrak{P}$
Phenylacetic acid (2 vols. xylene to 1 vol. of water)	3.575	4.825	1.35	—
	2.875	3.325	1.16	P[$C_2 = 2m$ L.]
	2.250	2.400	1.068	
	1.400	1.275	0.910	1.02
Phenylacetic acid (2 vols. water to 1 vol. xylene)	4.90	3.05	0.623	—
	2.58	0.85	0.330	P[$C_2 = 0.5m$ L.]
	1.75	0.35	0.200	—
	1.18	0.175	0.148	0.253
Phthalic acid	38.60	1.70	0.0430	—
	32.55	1.25	0.0384	—
	14.45	0.35	0.0243	0.0355
Piperonylic acid	0.865	1.14	1.32	—
	0.660	0.779	1.182	—
	0.3595	0.3142	0.875	1.29
Propionic acid	19.30	1.30	0.067	—
	9.20	0.60	0.065	—
	3.80	0.20	0.0528	0.066
Pyruvic acid	63.7	1.85	0.029	—
	45.8	1.10	0.024	—
	19.6	0.40	0.0204	0.0235
Resorcinilic acid	40.5	1.50	0.037	—
	26.8	0.80	0.0298	—
	19.2	0.50	0.0260	0.032
Salicylic acid	4.675	5.675	1.215	—
	3.075	3.075	1.000	—
	1.785	1.238	0.692	—
	1.350	0.862	0.638	—
	1.038	0.550	0.530	0.650
n-Valeric acid	8.40	11.6	1.38	—
	5.00	5.00	1.00	—
	2.30	1.70	0.74	—
	2.175	1.575	0.725	—
	1.500	0.925	0.616	—
	1.150	0.625	0.543	—
	0.6625	0.3125	0.472	0.635
iso-Valeric acid	6.15	3.15	0.512	—
	5.30	2.60	0.490	—
	4.275	1.925	0.450	—
	3.85	1.55	0.403	—
	2.35	0.875	0.372	—
	1.875	0.600	0.320	0.370

Section II *(Continued)*

The Partition Coefficients of the Bases between Water and Xylene, 25°

	C_1	C_2	C_2/C_1	$\mathfrak{P}$
Allylamine	6.50	1.30	0.200	—
	3.30	0.55	0.196	—
	1.85	0.35	0.189	0.198
n-Amylamine	5.08	14.92	2.94	—
	2.655	7.17	2.70	—
	1.875	4.275	2.28	—
	1.400	3.05	2.18	—
	1.335	2.67	2.00	—
	0.755	1.15	1.52	1.46
iso-Amylamine	2.65	5.10	1.92	—
	1.00	1.50	1.50	—
	0.80	1.175	1.47	—
	0.75	1.073	1.43	—
	0.55	0.700	1.28	—
	0.525	0.650	1.24	1.38
Anilin	0.378	2.00	5.3	—
	0.279	1.20	4.3	—
	0.203	0.67	3.3	—
	0.150	0.45	2.9	4.00
Benzylamine	6.32	13.68	2.16	—
	3.35	6.65	1.99	—
	1.58	2.42	1.53	—
	0.81	1.19	1.47	1.40
Benzylethylamine	1.40	18.60	13.4	—
	0.70	9.30	13.3	—
	0.35	4.65	13.3	—
	0.141	1.86	13.5	—
	0.103	1.368	13.6	13.4
Benzylmethylamine	1.138	8.86	7.80	—
	0.75	5.00	6.66	—
	0.226	1.20	5.30	—
	0.15	0.725	4.83	5.10
Bornylamine	0.08125	4.72	58.00	—
	0.0570	3.16	55.50	—
	0.0455	2.50	55.00	ca. 54.00
n-Butylamine	5.60	4.40	0.785	—
	2.40	1.50	0.665	—
	1.28	0.718	0.560	—
	0.671	0.328	0.490	0.60

SECTION II (*Continued*)

	C_1	C_2	C_2/C_1	$\mathfrak{p}$
iso-Butylamine	3.575	2.075	0.58	—
	1.775	0.975	0.55	—
	0.625	0.362	0.58	0.58
Camphylamine	0.0625	3.587	57.9	—
	0.0396	2.210	55.8	—
	0.0182	0.982	54.0	ca. 54.2
Coniine	0.935	9.065	9.7	—
	0.495	3.505	7.1	—
	0.265	1.735	6.5	—
	0.146	0.861	5.9	6.0
Diethylamine	12.68	7.42	0.59	—
	6.45	3.55	0.552	—
	4.075	1.925	0.472	—
	2.950	1.30	0.441	—
	2.72	1.28	0.470	—
	1.95	0.75	0.410	—
	1.45	0.55	0.380	0.44
Dimethylamine (in water)	17.78	2.22	0.125	—
	8.97	1.12	0.125	—
	3.55	0.430	0.121	—
	1.786	0.214	0.120	0.120
Dipropylamine	2.42	17.58	7.25	—
	1.45	8.55	5.90	—
	0.85	4.15	4.88	—
	0.575	2.325	4.04	—
	0.456	1.544	3.38	—
	0.425	1.525	3.59	—
	0.323	1.04	3.19	3.15
Ethylamine (in alcohol)	19.284	1.426	0.074	—
	9.255	0.745	0.0805	—
	3.654	0.346	0.0947	—
	1.7955	0.2045	0.1140	0.077
n-Hexylamine	2.80	17.2	6.15	—
	1.50	8.50	5.65	—
	0.7775	3.225	4.17	—
	0.625	2.325	3.88	—
	0.425	1.3375	3.14	—
	0.225	0.5875	2.61	3.05
n-Heptylamine	0.445	9.565	21.5	—
	0.438	9.562	21.8	—
	0.270	3.730	13.8	—
	0.152	1.848	12.3	—
	0.083	0.917	11.0	11.2

SECTION II (*Continued*)

	C_1	C_2	C_2/C_1	$\mathfrak{p}$
Methylamine (in water)	19.018	0.982	0.0517	—
	0.499	0.501	0.0528	—
	3.745	0.255	0.0680	—
	1.730	0.130	0.0750	0.051
Methylanilin	0.300	3.40	11.3	—
	0.185	2.10	10.9	—
	0.125	1.31	10.5	10.45
n-Methylpiperidine	7.33	12.67	1.725	—
	3.70	6.21	1.640	—
	2.40	3.40	1.420	—
	1.85	2.575	1.390	—
	1.70	2.300	1.350	—
	1.05	1.325	1.260	—
	0.683	0.934	1.365	—
	0.700	0.9375	1.340	1.20
Phenylethylamine	0.4125	1.51	3.64	—
	0.3437	1.13	3.29	—
	0.2380	0.763	3.20	—
	0.1940	0.582	3.00	3.20
Piperidine	7.00	4.00	0.57	—
	3.725	1.975	0.53	—
	2.850	1.300	0.456	—
	2.500	1.200	0.480	—
	1.850	0.750	0.406	—
	1.25	0.450	0.360	0.440
Propylamine	7.75	2.25	0.290	—
	4.80	1.30	0.270	—
	2.45	0.60	0.245	0.26
Pyridine	9.75	19.00	1.95	—
	4.80	9.50	1.98	—
	2.05	3.80	1.85	—
	1.00	1.90	1.90	1.95
Quinoline	0.470	9.40	20.0	—
	0.250	4.20	16.8	—
	0.116	1.80	15.5	14.0
Triethylamine	2.53	17.47	6.90	—
	1.49	8.51	5.72	—
	0.80	3.60	4.50	—
	0.75	3.25	4.33	—
	0.625	2.475	3.96	—
	0.3875	1.412	3.57	—
	0.3125	0.9875	3.16	3.17

SECTION II (*Continued*)

	C_1	C_2	C_2 C_1	$\mathfrak{p}$
Trimethylamine (in alcohol)	14.22	5.78	0.407	—
	6.97	3.03	0.435	—
	2.775	1.225	0.442	—
	2.13	1.00	0.47	0.47
Xylidene, 1, 3, 4	0.2875	3.3125	11.5	—
	0.1385	1.525	11.0	—
	0.1070	1.200	11.2	11.0
Xylidene, 1, 4, 3	0.2875	4.462	15.5	—
	0.1420	2.150	15.1	—
	0.0740	1.100	14.9	14.9
The Partition Coefficients of Acids between Water and Chloroform, 25°				
Acetic acid	20.9	2.00	0.0955	—
	13.8	1.00	0.0725	—
	8.35	0.45	0.0540	—
	5.85	0.235	0.0400	0.0725
Anisic acid	0.225	1.75	6.36	—
	0.206	1.206	5.85	—
	0.162	0.875	5.38	—
	0.125	0.625	5.00	5.50
Anthranilic acid	16.10	2.87	0.178	—
	10.00	1.54	0.154	—
	6.62	0.96	0.145	—
	4.50	0.60	0.133	0.148
Benzilic acid	0.640	1.75	2.74	—
	0.460	0.96	2.08	—
	0.2305	0.2885	1.25	2.12
Benzoic acid	1.137	4.55	3.96	—
	0.675	2.125	3.15	—
	0.599	1.84	3.07	—
	0.396	1.054	2.66	—
	0.3025	0.732	2.42	—
	0.2380	0.5625	2.36	2.65
Bromoacetic acid	32.00	2.08	0.065	—
	18.45	1.05	0.0570	—
	14.45	0.75	0.0519	—
	9.40	0.45	0.0478	0.0560
α-Bromopropionic acid	20.20	5.56	0.276	—
	13.00	3.48	0.268	—
	6.65	1.70	0.256	—
	4.40	1.10	0.250	0.248

SECTION II (*Continued*)

	C_1	C_2	C_2/C_1	$\mathfrak{p}$
n-Butyric acid	3.930	2.190	0.557	—
	2.290	1.252	0.546	—
	1.680	0.866	0.516	—
	1.402	0.710	0.506	—
	1.169	0.574	0.491	—
	1.040	0.488	0.470	0.530
sec.-Butyric acid	5.450	2.350	0.431	—
	3.190	1.275	0.400	—
	2.100	0.750	0.357	—
	1.025	0.350	0.342	0.385
Camphoric acid	19.25	10.35	0.538	—
	7.95	1.50	0.189	—
	4.30	0.525	0.116	0.120
n-Capronic acid	0.2125	1.4375	6.77	—
	0.1875	1.1375	6.08	—
	0.1158	0.6344	5.49	—
	0.0843	0.4281	5.07	6.1
iso-Capronic acid	0.4475	2.550	5.7	—
	0.2900	1.450	5.0	—
	0.2175	1.000	4.6	—
	0.1710	0.755	4.38	4.6
Chloroacetic acid	35.90	2.80	0.078	—
	28.60	1.55	0.0543	—
	16.10	0.50	0.0310	—
	12.00	0.30	0.0250	0.0438
o-Chlorobenzoic acid	1.075	2.690	2.50	—
	0.760	1.488	1.955	—
	0.649	1.072	1.655	—
	0.492	0.649	1.320	1.60
α-Crotonic acid	7.15	3.40	0.475	—
	5.10	2.125	0.417	—
	3.55	1.30	0.366	—
	2.35	0.75	0.319	0.340
α-*β*-Dibromopropionic acid	4.98	1.63	0.327	—
	3.54	0.985	0.278	—
	2.66	0.560	0.210	—
	1.85	0.313	0.169	0.280
2,4-Dinitrobenzoic acid	10.95	1.10	0.100	—
	8.60	0.70	0.0815	—
	7.85	0.55	0.070	—
	6.78	0.40	0.0590	0.10

SECTION II (*Continued*)

	C_1	C_2	C_2 C_1	𝔭
3,5-Dinitrobenzoic acid	4.04	3.71	0.918	—
	2.855	2.38	0.834	—
	1.992	1.425	0.715	—
	1.450	0.975	0.672	0.68
Formic acid (average value)	32.2	1.00	0.031	0.031
Gentisinic acid	38.40	0.90	0.0234	—
	20.70	0.45	0.0217	—
	15.20	0.32	0.0210	0.0236
Glutaric acid	33.1	0.905	0.0273	—
	24.70	0.570	0.0231	—
	17.90	0.375	0.0210	0.029
Hydrocinnamic acid	0.177	1.770	10.00	—
	0.134	1.190	8.87	—
	0.1058	0.835	7.90	—
	0.089	0.650	7.30	8.40
o-Iodobenzoic acid	0.473	1.990	4.21	—
	0.335	0.973	2.90	—
	0.287	0.680	2.37	—
	0.244	0.493	2.02	—
	0.248	0.512	2.06	2.92
β-Iodopropionic acid	5.55	3.50	0.630	—
	3.70	2.12	0.572	—
	2.80	1.542	0.552	—
	1.61	0.805	0.500	—
	1.162	0.5375	0.462	0.515
Lactic acid	79.8	1.80	0.0226	—
	50.4	1.00	0.0198	—
	32.8	0.60	0.0183	—
	26.9	0.43	0.0160	0.0198
Laevulinic acid	24.5	2.00	0.0817	
	18.55	1.35	0.0728	
	12.80	0.75	0.0586	0.063
o-Nitrobenzoic acid	8.65	3.35	0.388	—
	6.60	2.05	0.310	—
	5.55	1.55	0.279	—
	2.775	0.45	0.162	0.225
m-Nitrobenzoic acid	0.950	1.625	1.71	—
	0.675	0.975	1.44	—
	0.4874	0.600	1.23	1.46

SECTION II (*Continued*).

	C_1	C_2	C_2/C_1	$\mathfrak{p}$
p-Nitrobenzoic acid	1.05	1.975	1.88	
	0.75	1.175	1.57	—
	0.525	0.687	1.31	—
	0.364	0.400	1.10	1.50
Phenylacetic acid	0.715	1.542	2.16	—
	0.518	1.010	1.95	—
	0.425	0.785	1.845	—
	0.280	0.475	1.70	1.95
Piperonylic acid	0.250	1.0375	4.15	—
	0.2187	0.8125	3.71	—
	0.175	0.5812	3.32	4.00
Propionic acid	10.75	1.95	0.181	—
	7.12	1.175	0.165	—
	2.92	0.40	0.137	—
	5.65	0.85	0.153	0.163
Pyruvic acid	31.2	2.00	0.064	—
	16.90	1.00	0.0592	0.0592
Salicylic acid	2.625	6.575	2.50	—
	1.425	2.625	1.84	—
	1.138	1.862	1.64	—
	0.850	1.180	1.39	—
	0.608	0.730	1.20	—
	0.575	0.625	1.09	1.30
Succinic acid	48.00	0.95	0.0198	—
	34.90	0.60	0.0172	—
	20.50	0.30	0.0146	—
	13.50	0.15	0.0110	0.020
Valeric acid	1.825	4.625	2.53	—
	0.910	2.00	2.20	—
	0.650	1.35	2.08	—
	0.890	0.70	1.80	1.90
Valeric acid	3.35	4.55	1.36	—
	2.45	2.90	1.182	—
	1.585	1.65	1.040	—
	1.230	1.162	0.94	0.91

The Partition Coefficients of the Bases between Water and Chloroform, 25

i-Amylamine	0.2000	2.30	11.5	—
	0.1735	1.70	9.8	—
	0.150	1.20	8.0	—
	0.1168	0.6832	5.86	7.3

SECTION II (*Continued*)

	C_1	C_2	C_2/C_1	μ
Benzylamine	1.20	19.6	16.3	
	0.65	9.55	14.7	
	0.30	3.85	12.9	10.5
Diethylamine	4.350	17.95	4.13	
	1.625	5.025	3.09	
	0.800	2.000	2.50	
	0.300	0.600	2.00	2.20
Dimethylamine (in water)	5.85	3.40	0.582	
	4.55	2.65	0.582	
	3.40	2.00	0.588	
	2.20	1.275	0.580	
	1.35	0.80	0.592	0.588
Ethylamine	25.50	20.70	0.78	
	18.45	9.45	0.512	
	10.20	4.80	0.470	
	4.30	1.80	0.418	
	2.525	1.00	0.396	
	1.50	0.55	0.366	0.396
Methylamine (in water)	27.4	4.5	0.164	
	13.5	1.9	0.141	
	6.15	1.225	0.199	Average
	2.625	0.475	0.181	0.171
Triethylamine	0.425	9.575	22.5	
	0.194	3.806	19.7	
	0.0516	0.800	15.5	
	0.0375	0.490	13.0	16.0
Trimethylamine (in alcohol)	4.85	14.95	3.08	
	1.05	2.90	2.62	
	0.60	1.50	2.50	
	0.30	0.70	2.33	2.58
Piperidine	1.725	6.225	3.61	
	1.200	3.625	3.02	
	0.643	1.556	2.42	
	0.400	0.800	2.00	2.18
Pyridine	1.45	16.25	11.2	
	0.55	5.10	9.3	
	0.30	2.2	7.3	
	0.225	1.225	5.4	5.4

§3. Discussion

In the first part of this paper the generalized concepts of molecular volume and secondary valence were discussed in some detail. On the assumption that molecular volume is one of the essential or determining factors in all intermolecular relations of liquids we are going to use it in the analysis of the relations involved in partition coefficients.

The choice between molecular volume and molecular weight is not one which can be settled by literal proof. In the end it is a matter of interpretation and a decision must rest upon the relative value of the results to be obtained by the use of each. The reader is urged to keep in mind that such disclosures as are made through the use of molecular volume would not appear were one considering molecular weight; in fact, there would be no intelligible relationship whatever. This is especially true when we consider compounds containing halogens, ring formations, hydroxyl groups, ethylene linkages, etc., because then the relative differences in mass and volume become very great. It is a part of the purpose of this paper to establish the rôle of molecular volume in the intermolecular relations under consideration; and it is held that this rôle is established by the fact we are rewarded for its use with a significant simplification in these relations.

* * * * * * *

When we plot the logarithms of the partition coefficients of the amines and the acids into xylene against their respective molecular volumes we obtain the pictures reproduced in Figures I and II. Three outstanding facts are revealed by a glance at these pictures:

1. The observed points fall into several *series,* each of which is a straight line.
2. These *series* are all parallel.
3. The distances which separate the various *series* are related in a definite and periodic manner.

The equation for the straight line can be applied to any one of these *series* by extrapolating to $V_m = O$; with the constant of integration a thus obtained, the angular constant,

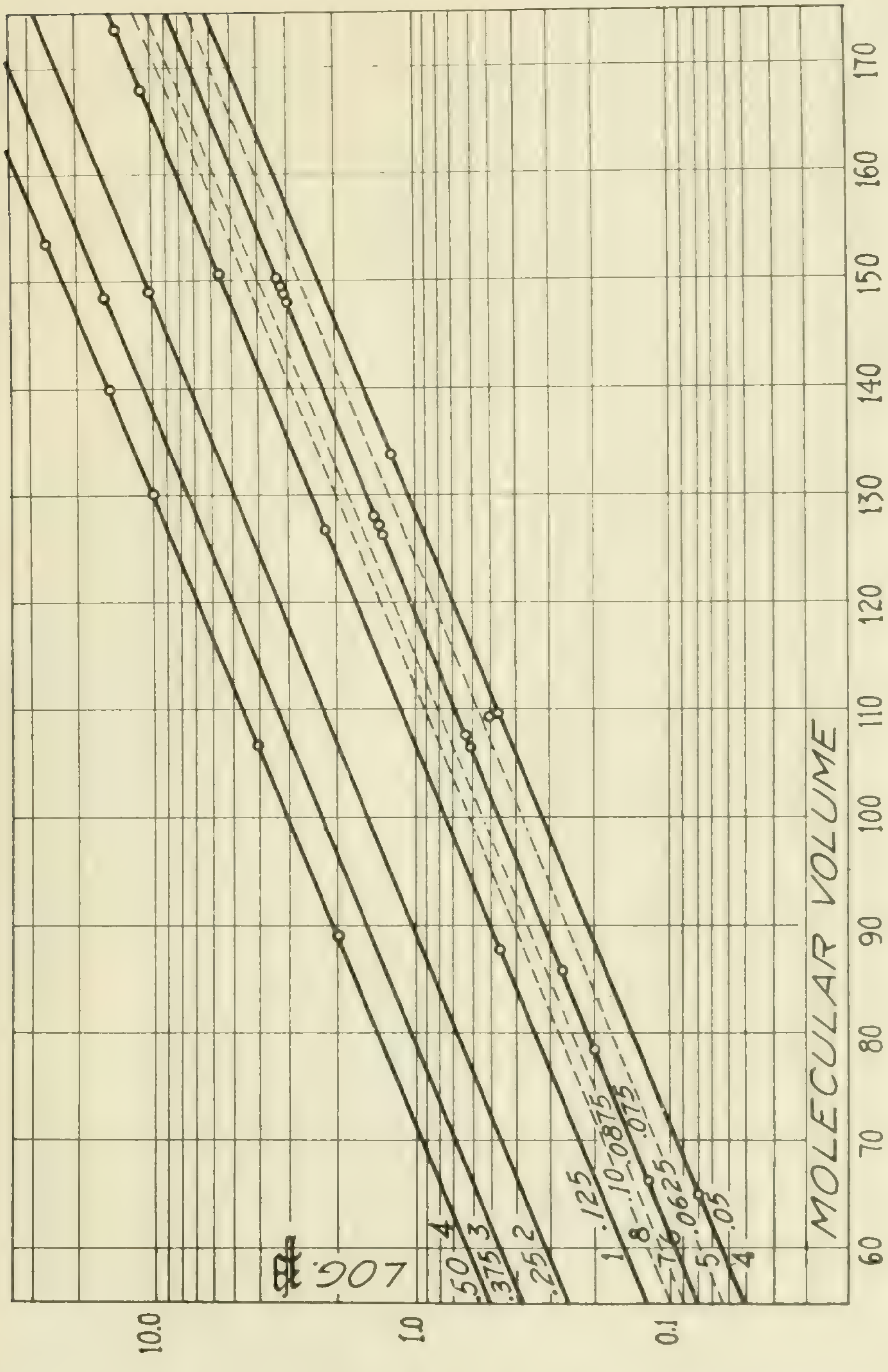

Fig. I
The Partition Coefficient of the Bases; Xylene

or constant of proportion, k can be calculated from the following equation:

$$\text{Log } 100\ \mathfrak{p} = \frac{V_m}{k} \pm a \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

On the basis of careful extrapolations the angular constant k has been given the value 60.00. This constant is common to all *series* since they are parallel.

The compounds which have been studied will be considered according to the *series* in which they fall. In the

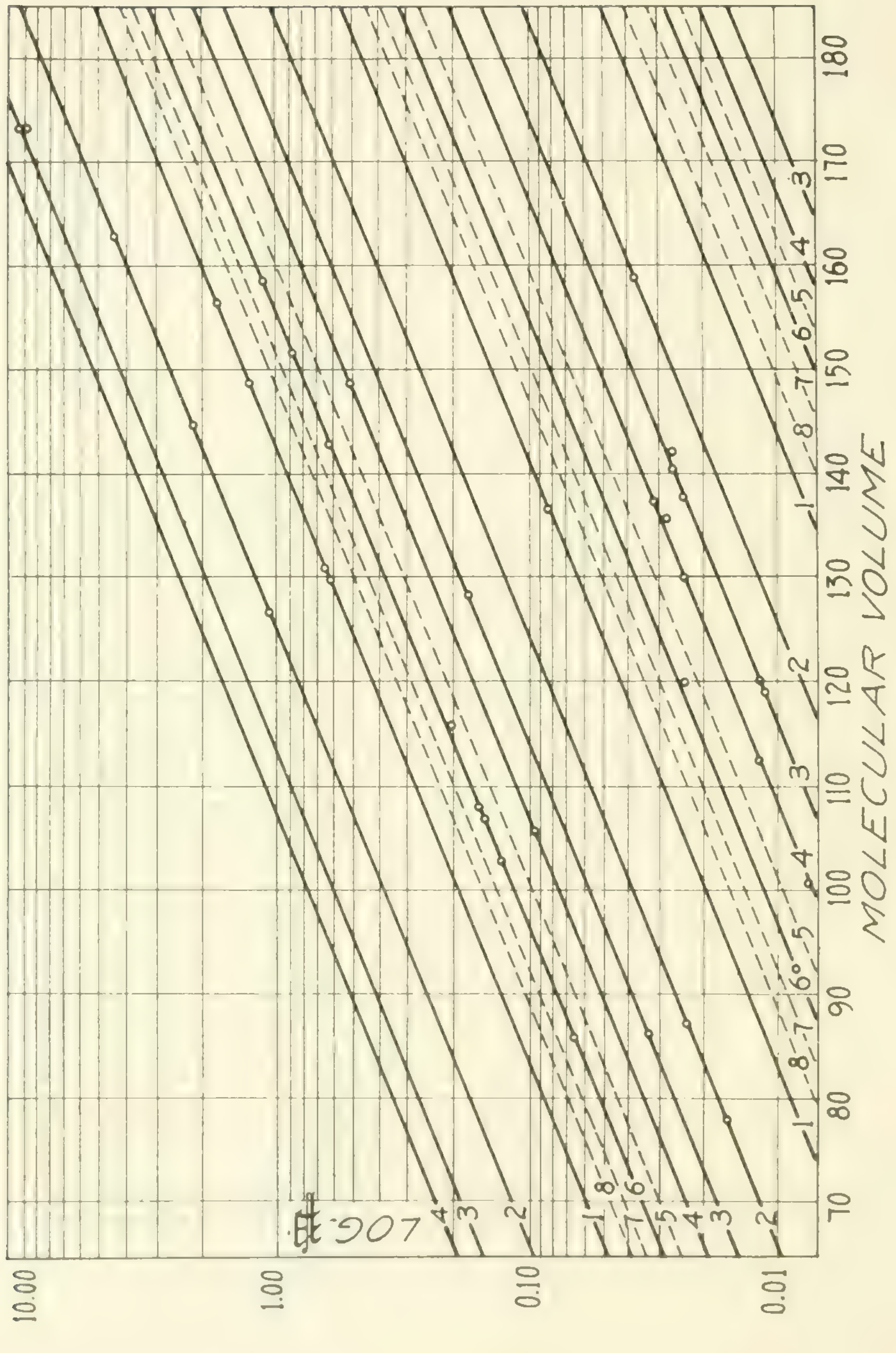

Fig. II
The Partition Coefficients of the Acids; Xylene

following tables the second column gives the molecular volumes; the italicized figures are observed values cited from Le Bas, and the others are calculated by Le Bas' rules. In column three, the osberved values of the partition coefficients 𝔓, are given and in the fourth column the values of 𝔓 as calculated from the equation given above using the *series* constant *1*. The ratios of the observed and calculated values in the fifth column are mathematical equivalents of statement 1 and 2 above.

TABLE I THE AMINES

The Partition Coefficients of the Bases, by Series; Xylene

	V_m	$\mathfrak{P}_{obs.}$	$\mathfrak{P}_{calc.}$	$\frac{\mathfrak{P}_{calc.}}{\mathfrak{P}_{obs.}}$
SERIES 4; $a = +0.8239$:				
Pyridine	89.5	1.95	2.07	106.1
Anilin	106.6	4.00	3.98	99.5
Methylanilin	129.6	10.45	9.19	114.0
Quinoline	140.0	14.0	14.36	102.4
Camphylamine	174.0	54.2	52.9	97.7
Bornylamine	174.0	54.0	52.9	97.9
SERIES 3; $a = +0.6989$:				
Xylidene, 1.4.3	148.6	14.9	14.98	100.4
SERIES 2; $a = +0.5229$:				
Xylidene, 1.3.4	148.6	11.0	10.00	90.8
SERIES 1; $a = +0.2218$:				
Trimethylamine	88.0	0.47	0.488	103.9
Benzylmethylamine	151.0	5.1	5.49	107.5
Heptylamine	169.5	11.2	11.14	99.6
Benzylethylamine	173.0	13.4	12.74	95.2
SERIES 8; $a = +0.1249$:				
SERIES 7; $a = +0.06695$:				
SERIES 6; $a = +0.0000$:				
Methylamine	43.0	0.051	0.052	102.0
Dimethylamine	66.3	0.125	0.127	101.8
Allylamine	78.5	0.198	0.203	102.5

TABLE I (*Continued*)

	V_m	$\mathfrak{P}_{obs}$	$\mathfrak{P}_{calc.}$	$\frac{\mathfrak{P}_{calc}}{\mathfrak{P}_{obs.}}$
Propylamine	*85.7*	0.26	0.268	103.0
iso Butylamine	*106.3*	0.58	0.591	101.9
n Butylamine	*106.8*	0.60	0.602	100.1
iso-Amylamine	*127.0*	1.38	1.310	95.0
Benzylamine	*127.4*	1.40	1.328	94.9
n Amylamine	*127.5*	(1.46)	1.340	(91.7)
n Hexylamine	*148.0*	3.05	2.925	96.0
Dipropylamine[1]	148.0?	3.15	(2.925)	(92.7)
	151.5		3.347	107.2
Triethylamine[1]	148.0?	3.17	(2.925)	(92.2)
	152.6		3.494	110.0
SERIES 5; a = 0.0792:				
SERIES 4; a = −0.1761:				
Ethylamine	*64.6*	0.077	0.0798	103.5
Piperidine	*108.8*	0.44	0.434	98.6
Diethylamine	*109.3*	0.44	0.442	100.2
Methylpiperidine	133.8	1.20	1.132	94.5
Coniine	*175.6*	6.0	5.63	94.0
				Av., 100.8

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[1] Both dipropylamine and triethylamine behave as though they had a volume nearly equivalent to hexylamine; secondary and tertiary nitrogen has a larger volume than primary nitrogen, and the low observed values may be due to impurities.

TABLE II—THE ACIDS

The Partition Coefficients of the Acids, by Series; Xylene

	V_m	$\mathfrak{P}_{\text{obs.}}$	$\mathfrak{P}_{\text{calc.}}$	$\frac{\mathfrak{P}_{\text{obs.}}}{\mathfrak{P}_{\text{calc.}}}$
SERIES 4; $a = +0.2354$:				
SERIES 3; $a = +0.1100$:				
iso-Amylacetic acid	174.1	10.7	10.40	97.3
Heptoic acid	174.6	10.0	10.48	104.8
SERIES 2; $a = -0.0655$:				
Benzoic acid	126.9	1.10	1.12	101.8
Cinnamic acid	162.7	4.5	4.43	98.5
SERIES 1; $a = -0.3666$:				
Valeric acid	130.0	0.635	0.631	99.5
Salicylic acid	130.6	0.650	0.646	99.5
Piperonylic acid	148.7	1.29	1.29	100.0
Anisic acid	156.5	1.80	1.75	97.2
Hydrocinnamic acid	170.1	2.75	2.94	106.9
SERIES 8; $a = -0.4634$:				
SERIES 7; $a = -0.5214$:				
SERIES 6; $a = -0.5884$:				
Formic acid	41.5	(0.0125)[1]	0.0125	100.0
Acetic acid	64.0	(0.030)[1]	0.030	100.0
Propionic acid	85.3	0.066	0.068	103.0
a-Crotonic acid	102.1	0.135	0.130	96.3
iso. Butyric acid	107.3	0.160	0.158	98.8
n Butyric acid	107.8	0.158	0.161	102.0

TABLE II (*Continued*)

	V_m	$\mathfrak{p}_{obs}$	$\mathfrak{p}_{calc}$	$\frac{\mathfrak{p}_{obs}}{\mathfrak{p}_{calc}}$
β-Iodopropionic acid	115.0	0.208	0.213	102.5
iso-Valeric acid	129.5	0.370	0.371	100.2
o-Chlorobenzoic acid	143.3	0.635	0.631	99.4
p-Nitrobenzoic acid	152.5	0.900	0.898	99.8
o-Iodobenzoic acid	159.4	1.15	1.17	101.8
SERIES 5; $a = -0.6676$:				
m-Nitrobenzoic acid	151.9	0.73	0.731	100.1
SERIES 4; $a = -0.7645$:				
α-Bromopropionic acid	105.8	0.095	0.099	104.2
Phenylacetic acid	148.9	0.51	0.521	102.2
SERIES 3; $a = -0.8894$:				
Bromoacetic acid	84.5	0.033	0.0333	100.0
α β-Dibromopropionic acid	127.8	0.168	0.174	103.5
SERIES 2; $a = -1.0655$:				
Acetic acid	64.0	(0.010)[2]	0.10	100.0
Chloroacetic acid	78.0	(0.0175)[1]	0.0172	98.3
Pyruvic acid	85.3	0.0235	0.0227	96.6
3,5 Dinitrobenzoic acid	175.8	0.70	0.732	104.5
SERIES 1; $a = -1.3665$:				
SERIES 8; $a = -1.4634$:				
SERIES 7; $a = -1.5214$:				

TABLE II (*Continued*)

	V_m	$\mathfrak{P}_{obs.}$	$\mathfrak{P}_{calc.}$	$\frac{\mathfrak{P}_{obs}}{\mathfrak{P}_{calc}}$
SERIES 6; $a = -1.5884$:				
Lactic acid	88.7	(0.00792)[3]	0.00774	97.7
o-Nitrobenzoic acid	150.8	0.085	0.0841	99.9
SERIES 5; $a = -1.6676$:				
SERIES 4; $a = -1.7645$:				
Oxalic acid	75.6	(0.003)[1]	0.0031	(103.3)
Malonic Acid	97.8	(0.0077)[1]	0.0073	(94.8)
Laevulinic acid	130.0	0.0242	0.0262	108.2
Itaconic acid	134.3	(0.0286)[1]	0.0298	(104.2)
Resorcinilic acid	136.4	0.032	0.0324	102.2
Acetylsalicylic acid	185.6	0.222	0.2132	96.0
SERIES 3; $a = -1.8894$:				
Succinic acid	120.0	(0.0123)[1]	0.0129	(104.8)
Gentisinic acid	136.5	0.0242	0.0243	100.3
Bromosuccinic acid	140.5	0.0252	0.0283	112.3
Glutaric acid	142.2	(0.0253)[1]	0.0302	(119.2)
2,4 Dinitrobenzoic acid	175.3	0.100	0.1077	107.7
Pimelic acid	186.6	(0.139)[1]	0.166	(119.2)
Suberic acid	208.8	(0.345)[1]	0.389	(112.8)
Azalleic acid	231.0	(0.95)[1]	0.913	(95.1)

TABLE II (*Continued*)

	V_m	$\mathfrak{P}_{obs}$	$\mathfrak{P}_{calc.}$	$\frac{\mathfrak{P}_{obs}}{\mathfrak{P}_{calc}}$
SERIES 2; $a = -2.0655$:				
Maleic acid	112.6	(0.00668)[1]	0.00647	(96.8)
o-Phthalic acid	155.6	0.0355	0.0337	95.0
Benzilic acid	238.3	0.800	0.805	99.3
SERIES 1; $a = -2.3665$:				
SERIES 3, $a = -2.8894$:				106.2
Camphoric acid	215.7	0.048	0.051	
				Av., 101.4

NOTE In many instances the partition coefficient between water and xylene is too low to be determined directly. It is possible to calculate this partition coefficient if the probable ratio between $\mathfrak{P}$ xylene and $\mathfrak{P}$ for another solvent is known. This calculation gives only one of several possible values for $\mathfrak{P}$ xylene, and therefore figures so obtained cannot be used in making comparisons of the constitutive behavior of several compounds. But the fact that the values so obtained agree with the *Series* behavior is important, and so, in order to show this agreement and to complete the chemical groups in the above table, a few such calculated values have been included. For the derivation of the following ratios see §§4, 5, etc.

[2] Calculated from chloroform, ratio 2.5 to 1. §2, Sec. II.

[3] Calculated from toluene, ratio 1 to 1. Cf. H. and F., §2, Sec. I. Also from chloroform, ratio 2.5 to 1. Cf. D. and G. (20), §2, Sec. I.

[4] Calculated from chloroform, ratio 2.5 to 1.

[5] Calculated from ether, ratio 10 to 1. Cf. Chandler, §2, Sec. I.

The Role of Molecular Volume

The above tables, through the application of Equation 1, establish the fact that all these *series* are straight lines and parallel.

The function of molecular volume may be briefly stated as follows:

In any series, the partition coefficient is a simple logarithmic function of the molecular volume.

That is, for any change in molecular volume, there is a corresponding change on a percentage basis in the partition coefficient. Since the angular constant is the same for both the amines and acids, the function of molecular volume is the same in both cases.

The Dstance between Series

The most interesting aspect of these pictures is the distance which separates the various *series*. This distance is actually given by the respective values of a, as given in Tables I and II; but these values are logarithmic and their real significance is not evident until they are converted into their corresponding arithmetic values, or antilogs. Moreover, the values which have been given for a, and which are used in Equation I, are related to the particular ordinate: $V_m = O$, and would be numerically different were another volume chosen for comparison. So the universal relationship between the *series*, applying to any volume whatever, may be more easily understood if described in this manner:

The series delimit arithmetrically related intervals or increases in the partition coefficient. These intervals fall naturally into octaves, each octave containing eight intervals. Each octave comprises an increase of 10 times in the value of the partition coefficient. In terms of $\mathfrak{P}$, *the first seven intervals are arithmetrically equal; the eighth and last interval in any octave is arithmetrically twice the value of each of the preceding seven*

Since all experimental values fall upon one or the other of these *series*, the possible partition coefficients at any given volume are related to each other by definite multiples of a common factor. $\mathfrak{P}_1$, which is given numerically in the value, in terms of $\mathfrak{P}_1$, of the first series of any *octave* at that volume.

Suppose we choose in Figure I the ordinate: $V_m = 54.2$; then on this ordinate the possible partition coefficients are as follows:

	Possible partition coefficients	Series number
First octave	1.25	1
	2.50	2
	3.75	3
	5.00	4
	6.25	5
	7.50	6
	8.75	7
	10.00	8
	12.50	10
Second octave	(25.00)	(20)
	(37.50)	(30)

(This arrangement has been illustrated at the left-hand margin of Figure I.)

The Octaves

Thus in passing up a particular ordinate from $\mathfrak{P} = 1$ to $\mathfrak{P} = 10$, we cross nine *series* or eight *intervals*. The value of the ninth *series*, instead of being nine times 1.0, is ten times 1.0, thus beginning another *octave*. This ninth series completes a group of eight *intervals*, seven of which are arithmetically equal, each having a value of 1.0. The eighth *interval* has a value of 2.0 or twice the value of any *interval* in the preceding seven. A little consideration will show that this peculiar arrangement is nothing more than a consequence of dividing an increase of ten times in the value of $\mathfrak{P}$ into eight parts, when $\mathfrak{P}$ progresses logarithmically.

This may perhaps be better understood if we think of one *octave* as representing the total change which may take place in the molecule under given circumstances, and further suppose that the essential condition to be fulfilled is that an *octave* must end at ten times the value of $\mathfrak{P}$ at which it began.

The Series Numbers

From what has been said, it can be seen that, at any volume, the possible partition coefficients are related to each other as multiples of a common factor, and that the decimal value of the highest common factor depends simply upon the decimal value of $\mathfrak{P}$ under consideration. Now since the multiple number of any series, or its relative position in the *octave,* is always the same, the relative position of a particular *series* in any *octave* may be indicated by its multiple number; thus the *number* of any *series* refers to its position in the *octave,* but since all the *octaves* are identical in respect to the interrelations of the comprised *series*, any *series* may be considered irrespective of the particular *octave* in which it falls. In other words, *series 6* in one *octave* is comparable to *series 6* in any other *octave.*

The Importance of the Series Numbers

From the preceding paragraph it can be seen that each *series* possesses a characteristic individuality, so to speak, because of its position in the *octave.* That this characterization is of fundamental importance is borne out by a consideration of the constitutive relations disclosed in Table I and II.

Considering first Table I, we see that pyridine, aniline, methylaniline, quinoline, and collidin fall in *Series* 4; benzylmethylamine and benzylethylamine in *Series* 1; the majority of the aliphatic amines in *Series* 6, and the piperidine derivatives in *Series* 4. No general rules for constitutive behavior can be laid down, for there are obvious exceptions to the principle of chemically homologous groups, but despite the exceptions, one cannot but feel that chemical constitution is a very important factor in determining the *series* behavior.

Turning to Table II, we see that the aliphatic acids, up to and including iso-valeric, fall in *Series 6* (cf. aliphatic amines, also *Series* 6). Valeric acid slips from *Series* 6 to *Series* 1, and heptoic acid to *Series* 3. Caproic acid should presumably fall in *Series* 2.[1] The majority of the dibasic acids fall in *Series* 3. These are only a few of the many instances which might be discussed in connection with constitutive behavior, but they are sufficient to show that:

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[1] See footnote, p. 245.

The number of the series in which a compound falls is indicative of some fundamental property of that particular molecular species under the given conditions.

The Blank Seres

If we summarize the distribution of compounds by *Series*, we see that the vast majority of them fall in *Series* 1, 2, 3, 4 and 6. (In the following table, the partition coefficients of the amines and acids between water and chloroform have been included. These partition coefficients will be considered in detail later on.)

Series number	No. of observed cases
1	19
2	17
3	23
4	22
5	(1)?
6	35
7	None
8	(1)?

This uneven distribution in the *octave* is emphasized by the behavior of chemically related compounds. Attention has already been called to the behavior of valeric acid, which left the company of formic, acetic, propionic, butyric and isovaleric acid in *Series* 6 to go into *Series* 1. It might have gone into *Series* 7 or 8, but it didn't. Again in the amines, benzylamine appears in *Series* 6, with the aliphatic amines, while benzylmethylamine and benzylethylamine avoid *Series* 7 and 8 to appear in *Series* 1. Trimethylamine and heptylamine also leave the other aliphatic amines in *Series* 6, to appear in *Series* 1. There are numerous other instances which might be mentioned to show that when a compound deviates from the behavior of allied compounds, it carefully avoids these blank *Series*.

The fact that there are so very few instances of compounds falling in *Series* 5, 7 or 8, suggests that the two which have been so far observed may be regarded somewhat as anomalies.[1] But since there is no apparent reason why compounds should not fall in these *Series* the time has not yet come to worry about them. The fact remains that *Series* 5, 7 and 8 appear to represent impossible or very improbable conditions, and this peculiar distribution in the *octave* may turn out to be significant in the final consideration of the underlying mechanism.

Some General Considerations

Secondary valence has already been defined as the force which binds molecules together. As a first step in the simplification of our problem, it was assumed that there is only one force involved in this union. It was pointed out that under this supposition, it was necessary to assume further that this force depended to a very great extent upon the chemical constitution of the molecule.

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[1] The exceptions to this distribution in *Series* 1, 2, 3, 4 and 6 are:

	Series No.	$\mathfrak{P}_{\text{obs}}$	$\mathfrak{P}_{\text{calc}}$
m-Nitrobenzoic acid (xylene)	5	0.73	0.73
n-Caproic acid (chloroform)	8	6.0	6.27
n-Caproic acid (xylene)	?	2.37	?
iso-Caporic acid (xylene)	?	1.75	?

m-Nitrobenzoic acid properly appears in *Series* 2 in chloroform and *iso*-caporic acid in *Series* 6; the former was repeatedly recrystallized and there can be no question of error in the partition coefficient for the sample investigated. The caproic acids fall between *Series* 1 and 2 in xylene; it is interesting to note that their relative values are the same in both solvents:

$$\text{Xylene}\quad \frac{\text{Caproic acid}\quad 2.37}{\textit{iso}\text{-Caproic acid}\quad 1.75}\quad 1.35$$

$$\text{Chloroform}\quad \frac{\text{Caproic acid}\quad 6.1}{\textit{iso}\text{-Caproic acid}\quad 4.6}\quad 1.32$$

It was impossible to redistill either of the small samples of these compounds available, but it is hoped that new samples will correct this anomalous behavior.

The partition coefficient is an index of the relative solubility of a substance in the two solvents, and consequently it is an index of the extent of the forces involved in the process of solution. We have just seen that the partition coefficient depends upon two factors: one is molecular volume; the second factor, made evident in the rhythmic variation in the partition coefficient, can only be construed to indicate abrupt changes in the intensity of secondary valence characterizing the various molecular species. Since the rôle of molecular volume is identical in all cases, it is obligatory to assume that those compounds falling in a particular *series* have a common *intrinsic* intensity of secondary valence. This deduction is supported by the constitutive relations previously discussed; for if chemical constitution has anything to do with the intensity of secondary valence, we should certainly expect chemically homologous groups, such as the anilines, the aliphatic amines, the piperidines and the aliphatic acids to behave in a similar fashion.

If, then, compounds falling in the same *series* are alike in regard to secondary valence, the separation of the various *series* by definite distances must mean that.

The characteristic intensity of secondary valence in various molecular species differs by definite and related amounts.

In Part I, it was shown how the intensity of secondary valence associated with any molecular species depended upon the arrangement of the atoms in the molecule. These constitutive relations concern—and this is extremely important only one or two atoms in the entire molecule. Yet these one or two atoms can endow the molecule with its characteristic secondary valence and thereby determine the molecular behavior. Such atoms (of which oxygen and nitrogen are the most important) may themselves vary in activity between wide extremes. Any variations relating to comparative molecular behavior must be attributed to changes in these particular atoms. (In the amines, it is obviously the nitrogen which is concerned; in the acids, it is principally the hydroxylic oxygen.) Now this rhythmic variation in secondary valence is displayed by all types of compounds derived from amino nitrogen and acidic oxygen; the value of the unit increase is the same in every case, and the peculiar distribution in the *octave* (*i. e.*, in *Series* 1, 2, 3, 4 and 6) likewise relates to all the compounds so far studied. These facts lead us to an extremely important deduction, namely:

Secondary valence must be attributed to some part of the atom which is identical in its nature in, and common to, all atomic species.

The great susceptibility of secondary valence to constitutive relations and the fact that in some atoms it varies between wide extremes shows us that:

This atomic mechanism may vary in some atoms in its ability to make itself felt. It functions, so to speak, in a rhythmic fashion, and its activity is determined by the mode of chemical combination.

Since all atomic species may exist in the liquid state, *all atomic species must possess some secondary valence.* It is not possible to state any relation between this minimum, or basal, elementary value and the value which a given species will have when combined with other atoms in the molecule; so it may be necessary to consider ultimately both a basal elementary value and a basal atomic value in the state of chemical combination with other elements. In any event, it is probable that the fluctuations which we have been observing are in addition to this basal atomic value. Now the question arises, what are the limits to the fluctuations in secondary valence associated with any one atom? Examination of the available data shows that compounds containing only one atom characterized by variable activity, such as oxygen and nitrogen, are always encompassed within one *octave*. It is only when two such atoms are present in the molecule, as in the hydroxy acids, the dicarboxylic acids, etc., that the compound is found in the second *octave*, or more than eight intervals removed from any compound containing but one variable atom. This fact, together with the repetitive and self-contained nature of the *octave* and its exact arithmetic equivalence to an increase in the partition coefficient of ten times, leads to the supposition that *one atom may furnish, in addition to its basal atomic value, one octave, or eight units of secondary valence. Two atoms may furnish presumably as many as sixteen, though constitutive interactions usually reduce the net amount.*

It does not follow that any atom will vary in this manner; carbon and hydrogen appear to possess a constant and low value.

If all the molecules under consideration contained only one variable atom, these molecules would all fall within the limits of one *octave.* But many compounds have been included in this study which contain several such atoms; hence these compounds are found scattered through two and even three *octaves.* Each successive *octave* must be looked upon as complete, however, though possibly adjoining other *octaves* by virtue of the superimposition of the last *series* of one on the first *series* of the other.

The Relation between Secondary Valence and Atomic Volume

It has long been recognized that atomic volumes are variable, and that the mode of chemical combination is the cause of these variations. Since there is a close relationship between the valence of an element and its elementary atomic volume, it is not surprising that the mode of chemical combination should influence the atomic volume in the combined state. We know practically nothing about the mechanism which underlies the variations in primary valence unions—those complex relations which G. N. Lewis has brought together under the term "polarity" and it is impossible at the present time to quantitate these "polar-volume" relations. These atomic volume relations appear, however, to be of great importance in connection with the nature of secondary valence. Students of molecular physics, from Kopp's time on, have sensed a relation between atomic volume and secondary valence. What we are now calling secondary valence has been called by divers authors "unsaturation," "subsidiary" and "residual valences," etc. The view a particular author took of this atomic mystery depended to a great extent upon the type of phenomena in which he was particularly interested. But it has been a common opinion that the "activity" of "unsaturated atoms" increased with decreasing volume. This is nicely illustrated in the behavior of oxygen. If we compare the boiling points and the solubility in water of an ether, an alcohol, an acid, and an ester, we see the close parallel which exists between the molecular behavior and the volume of the hydroxylic oxygen [Va O′].

	V_m	Va O′	B. P.	Sol. in 100 pts. water, 20
$C_2H_5OC_2H_5$	106.4	11.0	35.0°	7.30
$CH_3COOC_2H_5$	106.1	9.0	77.0	8.36
C_4H_9OH	102.1	6.4	116.8	8.35
C_3H_7COOH	107.8	6.4	163.0	20.00

Or, if we wish to compare various atomic species, the alkali metals are a classical example. The atomic volume increases in the order $Li < Na < K < Rb < Cs$. The salts of lithium are the most hydroscopic and the salts of caesium the least, thus indicating that the atoms with the smaller atomic volumes have the greatest affinity for water. The ionic velocity varies in the same order, lithium being the slowest and rubidium and caesium the fastest. The work of Jones and his collaborators showed that lithium was the most hydrated in aqueous solution, and caesium the least. In view of the facts that a particular atomic species varies in secondary valence according to the mode of chemical combination and that there are simultaneous changes in atomic volume which appear to be closely related to these secondary valence changes, the following assumption, though tentative, may aid us in the further analysis of the mechanism underlying atomic state:

The secondary valence associated with a particular atom in the combined state varies inversely as the volume of the atom;

the atomic volume, as well as the secondary valence, depends upon the mode of chemical combination.

§4. The Partition Coefficients into Chloroform

The partition coefficients of the acids and amines into chloroform have been analyzed in the same manner as were those into xylene. A comparison of Figure III with Figures I and II will show that the gross relations are identical. The angular constant, k, is the same in all cases, *i. e.*, 60.00, and hence the chloroform *series* are parallel to the xylene *series*. The inter-relations of *series* are the same so far as general proportionality is concerned, though the absolute values of a differ; in chloroform, P for a particular *series* at a given volume has just five times the value that it has in xylene.

In Tables III and IV the partition coefficients into chloroform have been tabulated just as were the partition coefficients into xylene. The calculated values given in these tables are derived through Equation I, using the *series* constants a as indicated, and the universal constant $k = 60.00$. The agreement between the observed and calculated values shows that the chloroform series constitute straight and parallel lines; since the *series* constants are based upon the ideal existence of *octaves* with related *series* as found in the case of xylene, it can readily be seen that there is no essential difference in the fundamental relationships in xylene and chloroform. The distribution of compounds by *series* has already been given in the previous section.

Partition Coefficient Ratios

One of the conditions imposed upon our method of correction was that the ratio $\frac{\mathfrak{P}_{\text{chloroform}}}{\mathfrak{P}_{\text{xylene}}}$ should be a constant. We can see now how this comes about. If we suppose that every compound falls in the same *series* in chloroform as it did in xylene, the ratio between the partition coefficients into the two solvents should always be 5.0, because, as has been said, a particular *series* in chloroform has a value of $\mathfrak{P}$ at a given volume just five times its value at that same volume in xylene. But from Table V we see that this ratio is not always

TABLE III

The Partition Coefficients of the Bases, by Series; Chloroform

	V_m	$\mathfrak{P}_{obs.}$	$\mathfrak{P}_{calc.}$	$\frac{\mathfrak{P}_{obs.}}{\mathfrak{P}_{calc.}}$
SERIES 2; $a = +0.2219$:				
Pyridine	*89.5*	5.1	5.17	101.2
SERIES 1; $a = \quad 0.1249$:				
Trimethylamine	*88.0*	2.38	2.197	92.3
Benzylamine	*127.4*	10.5	9.96	94.9
SERIES 8; $a = -0.2219$:				
SERIES 7; $a = -0.2799$:				
SERIES 6; $a = -0.3468$:				
Dimethylamine	*66.3*	0.588	0.573	97.5
n Amylamine	*127.5*	(7.30)[1]	6.00	(82.2)
Triethylamine	*152.6*	16.00	15.72	98.5
SERIES 5; $a = -0.4260$:				
SERIES 4; $a = -0.5229$:				
Methylamine	*43.0*	0.171	0.173	101.0
Ethylamine	*64.6*	0.396	0.397	100.2
Diethylamine	*109.3*	2.20	2.21	100.4
Piperidine	*108.8*	2.15	2.17	100.8
SERIES 3; $a = -0.6478$:				
SERIES 9; $a = \quad 0.8239$:				
				Av., 98.5

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[1] Note that in Table V the perfect ratio 5 indicates identical behavior in both xylene and chloroform; consequently the extraordinarily high value may reasonably be attributed to impurities.

TABLE IV

The Partition Coefficients of the Acids, by Series; Chloroform

	V_m	$\mathfrak{p}_{obs}$	$\mathfrak{p}_{calc}$	$\frac{\mathfrak{p}_{calc}}{\mathfrak{p}_{obs}}$
SERIES 3; a = +0.8094:				
SERIES 2; a = +0.6335:				
SERIES 1; a = +0.3324:				
Benzoic acid	*120.9*	2.65	2.80	105.8
SERIES 8; a = +0.2355:				
Caproic acid	*152.5*	6.10	6.27	102.8
SERIES 7; a = +0.1775:				
SERIES 6; a = +0.1100:				
n-Valeric acid	*130.0*	1.90	1.89	99.5
Piperonylic acid	148.7	4.00	3.87	96.8
iso-Caproic acid	152.0	4.60	4.39	95.5
Anisic acid	156.5	5.50	5.23	95.2
Hydrocinnamic acid	170.1	8.40	8.81	105.0
SERIES 5; a = +0.0308:				
SERIES 4; a = −0.0655:				
n-Butyric acid	*107.8*	0.530	0.540	102.0
Salicylic acid	130.6	1.30	1.29	99.3
SERIES 3; a = −0.1904:				
Formic acid	41.5	0.031	0.0317	102.2
Acetic acid	*64.0*	(0.0725)	0.0752	103.7
Propionic acid	*85.3*	0.163	0.170	104.3

TABLE IV (*Continued*)

	V_m	$\mathfrak{P}_{obs.}$	$\mathfrak{P}_{calc.}$	$\frac{\mathfrak{P}_{calc.}}{\mathfrak{P}_{obs.}}$
α-Crotonic acid	102.1	0.34	0.324	95.4
sec.-Butyric acid	107.3	0.385	0.396	102.9
β-Iodopropionic acid	115.0	0.515	0.532	103.2
iso-Valeric acid	129.5	0.91	0.928	102.0
o-Chlorobenzoic acid	143.3	1.60	1.577	98.6
Phenylacetic acid	148.9	1.95	1.955	100.2
o-Benzoic acid	159.4	2.92	2.926	100.2
SERIES 2; *a* = —0.3665:				
α-Bromopropionic acid	105.8	0.248	0.249	100.3
m-Nitrobenzoic acid	151.9	1.46	1.462	100.1
p-Nitrobenzoic acid	152.5	1.50	1.497	99.7
SERIES 1; *a* = —0.66756:				
Acetic acid	64.0	0.024	0.0251	104.6
Chloroacetic acid	78.0	0.0438	0.0429	97.9
Bromoacetic acid	84.5	0.0560	0.0550	98.2
Pyruvic acid	85.3	0.0592	0.0567	95.7
α-β-Dibromopropionic acid	127.8	0.280	0.290	103.6
SERIES 8; *a* = —0.7644:				
SERIES 7; *a* = —0.8224:				
SERIES 6; *a* = —0.8894:				
SERIES 5; *a* = —0.9686:				
SERIES 4; *a* = —1.0655:				
Anthranilic acid	135.0	0.148	0.153	03.3
3,5 Dinitrobenzoic acid	175.8	0.68	0.732	107.5

TABLE IV (*Continued*)

	V_m	$\mathfrak{p}_{obs}$	$\mathfrak{p}_{calc.}$	$\frac{\mathfrak{p}_{calc}}{\mathfrak{p}_{obs}}$
SERIES 3; *a* = 1.1904:				
Lactic acid	88.7	0.0198	0.0194	98.0
Nitrobenzoic acid	150.8	0.225	0.0210	93.4
SERIES 2, *a* = 1.3665:				
Laevulinic acid	130.0	0.063	0.0631	100.1
SERIES 1; *a* = 1.6675:				
Succinic acid	120.0	0.020	0.0215	107.7
Benzilic acid	238.3	2.12	2.01	94.8
SERIES 8, *a* = 1.7044.				
SERIES 7; *a* = 1.8224:				
SERIES 6, *a* = 1.8894:				
Gentisinic acid	136.5	0.0236	0.0243	103.0
Glutaric acid	142.2	0.029	0.0302	104.1
2,4-Dinitrobenzoic acid	175.3	0.100	0.1077	107.7
SERIES 5; *a* = 1.9686:				
SERIES 2; *a* = 2.3665:				
Camphoric acid	205.7	0.120	0.1153	96.3
				Av., 100.8
				Mean, 100.4

TABLE V

Partition Coefficients Ratios: $\frac{\text{Chloroform}}{\text{Xylene}}$

	$\mathfrak{p}_{\text{Xylene}}$	$\mathfrak{p}_{\text{Chloroform}}$	N_x	N_c	$\frac{\mathfrak{p}_{\text{Chloroform}}}{\mathfrak{p}_{\text{Xylene}}}$ or $5\frac{N_c}{N_x}$
Gentisinic acid	0.0242	0.0236	3	6	0.976
Bromoacetic acid	0.033	0.056	3	1	1.69
α β Dibromopropionic acid	0.168	0.280	3	1	1.66
p Nitrobenzoic acid	0.90	1.50	6	2	1.66
Succinic acid	(0.0123)[1]	0.020	3	1	1.66
Salicylic acid	0.650	1.30	1	4	2.0
Acetic acid	(0.010)[2]	(0.025)[3]	2	1	2.50
Benzoic acid	1.10	2.65	2	1	2.41
n. Butyric acid	0.160	0.385	6	3	2.41
β Bromopropionic acid	0.095	0.248	4	2	2.61
Camphoric acid	0.048	0.120	3	2	2.50
o Chlorobenzoic acid	0.635	1.60	6	3	2.52
α Crotonic acid	0.135	0.340	6	3	2.52
o Iodobenzoic acid	1.15	2.92	6	3	2.54
β Iodopropionic acid	0.208	0.515	6	3	2.48

[1] Calculated from ether, ratio 10 to 1, cf. §2. See I, Chandler

[2] Calculated from toluene, ratio 1 to 1, cf. §2. See I, H and F

[3] Cf. D₂, see I, chloroform, D and G

TABLE V (*Continued*)

	$\mathfrak{P}_{\text{Xylene}}$	$\mathfrak{P}_{\text{Chloroform}}$	N_x	N_c	$\frac{\mathfrak{P}_{\text{Chloroform}}}{\mathfrak{P}_{\text{Xylene}}}$ or $5\frac{N_c}{N_x}$
Laevulinic acid	0.0242	0.063	4	2	2.60
Propionic acid	0.066	0.163	6	3	2.47
Pyridine	1.95	5.1	4	2	2.62
Pyruvic acid	0.0235	0.0592	2	1	2.52
Anisic acid	1.80	5.50	1	6	3.06
Hydrocinnamic acid	2.75	8.40	1	6	3.05
Piperonylic acid	1.29	4.0	1	6	3.07
n-Valeric acid	0.635	1.9	1	6	2.99
n-Butyric acid	0.158	0.53	6	4	3.31
Methylamine	0.051	0.171	6	4	3.35
Phenylacetic acid	0.52	1.95	4	3	3.75
n-Amylamine	1.46	7.3	6	6	5.00
Diethylamine	0.44	2.2	4	4	5.00
Dimethylamine	0.12	0.588	6	6	4.90
Ethylamine	0.077	0.396	4	4	5.15
Piperidine	0.44	2.15	4	4	4.90
Triethylamine	3.17	16.00	6	6	5.07
Trimethylamine	0.47	2.38	1	1	5.06
Benzylamine	1.4	10.5	6	1	7.50

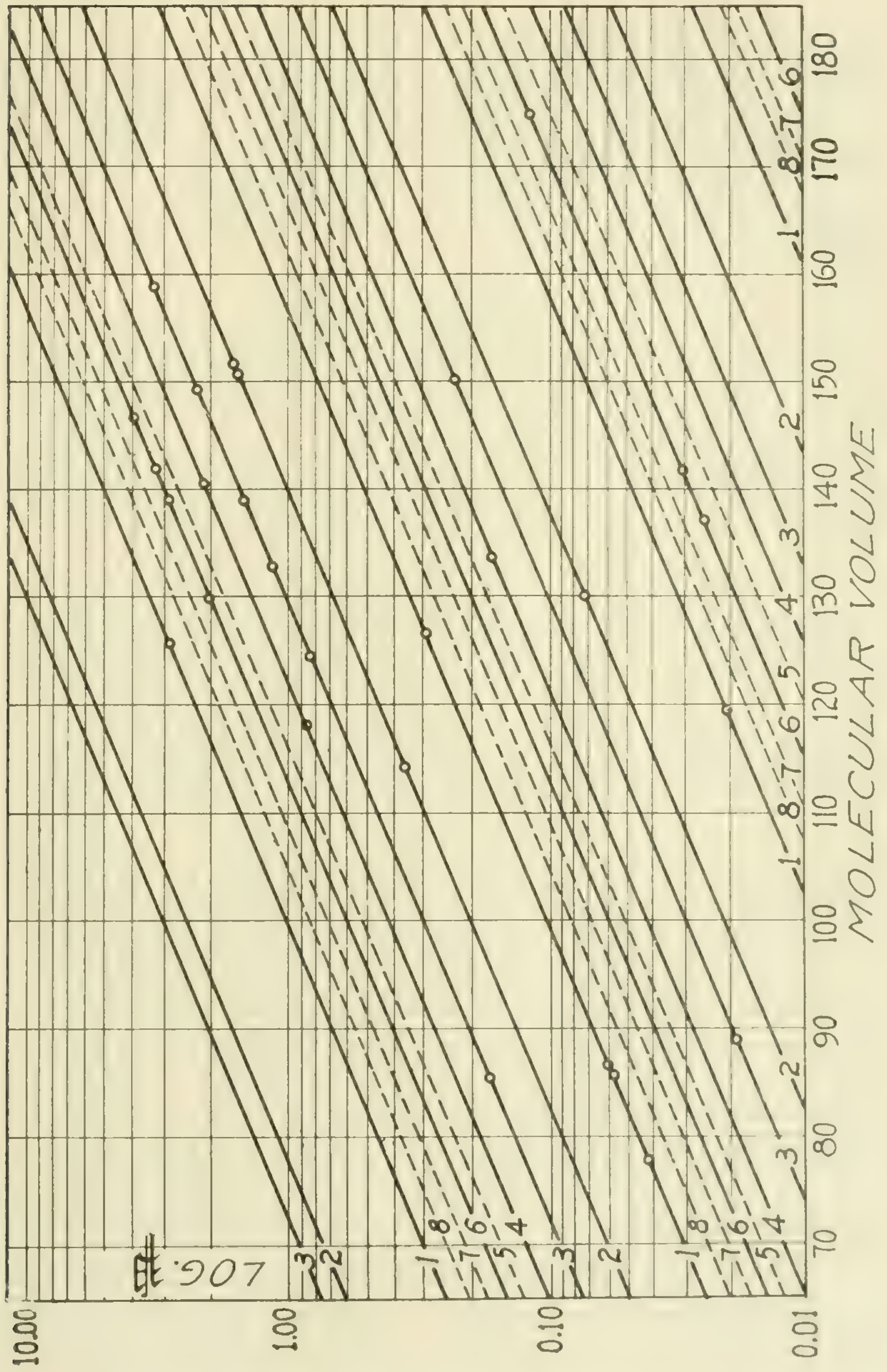

Fig. III

The Partition Coefficients of the Acids; Chloroform

5.0, but varies through a series of apparently related numbers running from 1.0 to 7.5. This is because compounds do not always fall in the same *series* in the two solvents. The possible ratios are limited, however, because the number of possible *series* is limited, and we may calculate the possible ratios on the basis that a compound may slip from any series in xylene to any series in chloroform. It is only necessary to consider one *octave*, for as a matter of fact, compounds never slip out of one *octave*. If we let n_x represent the *series* number in xylene, and n_c the *series* number in chloroform, the possible ratios are given by the formula

$$5\frac{n_c}{n_x} = r.$$

The 5 in the numerator is necessary because of the relative values of a particular *series* in the two solvents. The possible ratios have been tabulated below:

	n_x				
n_c	1	2	3	4	6
1	5.0	2.5	1.66	1.25	0.833
2	10.0	5.0	3.33	2.50	1.66
3	15.0	7.5	5.00	3.75	2.50
4	20.0	10.0	6.66	5.00	3.33
6	30.0	15.0	10.0	7.50	5.00

Space does not permit a discussion of all the constitutive relations involved in these partition coefficients ratios, but a few of the more important points need to be emphasized.

It will be recalled that most of the aliphatic amines and the unsubstituted aliphatic acids fell in *Series* 6 in xylene. In chloroform we find the amines in *Series* 6 again, hence the ratio 5.0; the acids, however, occur in *Series* 3, reducing the ratio 2.5. It was further pointed out that the acids from valeric on, in the case of xylene, slipped up a *series* with each succeeding member. In chloroform we see that this slipping process is repeated, but it begins with one member earlier in the group, namely, with butyric acid. Thus formic, acetic and propionic acids come in *Series* 3, butyric acid in *Series* 4, valeric acid *Series* 6. (Notice how valeric acid again avoids the blank *Series* 5.)

Several reasons have already been given for believing that these *series* numbers indicate the relative intrinsic intensity of secondary valence of the respective molecular species. This intensity, we have seen, depends upon the chemical constitution of the molecule and changes as a result of internal interactions between the component atoms. Now we see that this intensity is influenced not only by the chemical constitution of the molecule, but also by the environment. For though the same sample of butyric acid was used in determining the partition coefficient into xylene and chloroform, it does not behave in the same way towards the two solvents. In the former instance it fell in the same *series* as the preceding acids; in the latter instance, like valeric acid, it leaves the company of the preceding acids to pass into the next *series*. Numerous other instances of this same kind could be cited, all of which indicate some as yet obscure but definite principle underlying constitutive behavior. It is significant, however, that all these changes are in accordance with the *series* behavior, the *octave* distribution and some unformulated, yet obviously systematic relationship dependent upon chemical constitution.

But one important thing can be learned from these facts, and that is that *the intensity of secondary valence associated with a particular molecular species depends not only upon its chemical constitution, but also upon the nature of its environment.*

What constitutes Environment?

In both the systems which have thus far been considered, we have as components water and an immiscible liquid, with the solute distributed between them. It might be admitted that sufficient chloroform could dissolve in the water to cause these profound changes in the solute, but it is difficult to understand how xylene could exert any such influence in this manner for it is quite insoluble. But in both cases the second liquid is always intermolecularly connected, so to speak, with the water, through the interfaces and through the mutually contained solute. It must be through these channels, then, that equilibrium is established in such a system. The system consists of many molecular parts, each part capable of internal changes, evoked by external influences; every part in delicate equilibrium with every other part.

Thus it is necessary to recognize an "equilibrium in state" for liquids; an equilibrium between the electrons in the atom and the forces acting between molecules. If this equilibrium is disturbed at any point through the introduction of a molecule of a dissimilar substance, a strain will be transmitted throughout the system, affecting in turn every part. In view of these facts it is preposterous to suppose that the sole force involved in the distribution of a solute between two regions of a liquid, or between two immiscible liquids, is "gaseous diffusion pressure." The processes of diffusion and osmotic pressure appear to be consequences of the law of free energy working itself out through this molecule to molecule mechanism, distributing the strain in some definite manner in compliance with the nature of the electrical forces involved. At equilibrium the system must possess a homogeneity of state, if we may call it that, for otherwise under the law of free energy relations the dissimilar molecules would be forced to the interfaces, and out of the system.

The facts which have been presented in this paper throw some light on the relations of the solute to its environment, and on the question of what constitutes this environment. The nature of these relations is held to be the most important fact, apart from the rôle of molecular volume itself, disclosed by a study of partition coefficients:

In any liquid system, all the parts of which are in cohesive contact, there exist equilibrated intermolecular forces involving all the contained molecules.

These forces have been shown to vary in a rhythmic manner, the observed variations being attributable both to internal (constitutive) and to external (environmental) influences.

Thus a condition of equilibrium or compensation exists between the electrical mechanisms within the molecule (or its contained atoms) and the electrical forces which constitute its environment.

This equilibrium is not effected through gross electromagnetic laws, but through some as yet unpostulated rhythmic mechanism involving (1) the valence electrons; (2) the atomic volume?; (3) the intensity of secondary valence.

Summary

1. An empirical method has been given for eliminating the disturbing influences of dissociation, association, etc., in the distribution of a substance between two immiscible liquids. The validity of this method is substantiated by the fact that it discloses certain relationships between the partition coefficients of various compounds which appear to accord with well known laws pertaining to solutions.

2. The partition coefficients of a large number of organic acids and bases have been determined between water and xylene and between water and chloroform.

3. The relations between these partition coefficients have been analyzed in terms of molecular volume. The out-standing facts disclosed by this analysis may be summarized as follows:

A. The observed points fall into several series, each of which is a straight line.

B. These series are all parallel.

C. The distances which separate the various series are related to each other in a definite and periodic way.

4. The following relations appear in the more detailed analysis of the facts summarized in 3.

A. In any series of compounds having the same intrinsic intensity of secondary valence, the partition coefficient is a simple logarithmic function of the molecular volume.

B. Secondary valence is rhythmic in nature. That is, it varies in different molecular species by related units. These differences in secondary valence are expressed in the partition coefficient by increases or decreases of definite amounts.

C. These unitary changes in secondary valence are related to each other by octaves, one octave comprising an increase of ten times in the numerical value of the partition coefficient at any given molecular volume.

D. Each octave contains nine hypothetical series (see 3, A), representing eight unit increases in the partition coefficient.

E. The first seven of these steps, or unit increases, are arithmetically equal; the eighth is twice as large as any of the preceding seven. If we arbitrarily choose a volume at which the first series in the octave has a value in terms of $\mathfrak{P}$, of unity, then each of the first seven steps corresponds to an increase in the partition coefficient of an integral amount, or (1), 2, 3, 4 5, etc., the eighth step corresponds to an increase from 8 to 10; therefore the eight steps considered together (or one octave) corresponds to an increase from 1 to 10 or to an increase ten times in the value of $\mathfrak{P}$.

F. The relative position of a particular series in any octave may be indicated by its multiple number, or the number of times that the corresponding value of $\mathfrak{P}$, at a given volume, contains the highest common factor $\mathfrak{P}_1$ where $\mathfrak{P}_1$ represents the value of $\mathfrak{P}$ corresponding to the first series in the octave, at that volume. Thus the series numbers run from 1 to 8 inclusive.

G. Partition coefficients are observed in Series 1, 2, 3, 4 and 6; very rarely in 5, 7 or 8. (Evidence is considered in the text which indicates that Series 5, 7 and 8 probably represent unstable conditions.)

H. The differentiated nature of the octave, the peculiar distribution of compounds in the various series, and the principle of chemical homology which underlies this distribution by series all indicate that the series numbers have some very fundamental significance, in that they appear to represent some characteristic condition of the molecule as regards intensity of secondary valence.

5. Certain deductions of a more general nature have been based upon this analysis.

A. Since all the chemical compounds which have been studied so far behave in an identical manner so far as the foregoing points are concerned, it seems imperative to assume that secondary valence is to be attributed to some part of the atom which is identical in its nature in, and common to all atomic species.

B. The variable and rhythmic nature of secondary valence shows that this atomic mechanism may vary in some atoms in its ability to make itself felt; that it functions in a rhythmic fashion and that its activity is determined by the mode of chemical combination.

C. A study of constitutive relations shows that the variations attributable to one atom are confined to one octave, or eight unit changes.

6. The series number, which has been shown to be a fundamental characteristic (see 4 H) depends not only on the chemical nature of the compound, but also on the nature of its environment. This fact, coupled with the fact that molecular volume is a determining factor in intermolecular relations, leads to a deduction of fundamental importance in the theory of solution, namely:

In any liquid system, all the parts of which are in cohesive contact, there exist equilibrated intermolecular forces involving all the contained molecules. A condition of compensation exists between these intermolecular forces and the electrical mechanism in the molecule and its contained atoms in which they have their origin.

Baltimore, Md.

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THE CORRELATION BETWEEN MOTOR CONTROL AND RIFLE SHOOTING

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While carrying out a series of student experiments with the steadiness test as described by Whipple,[1] it occurred to one of us (D) that involuntary movements or tremors probably play an important, if not a predominant part in rifle shooting. To test the truth of this hypothesis we carried out a short series of preliminary experiments with a group of five soldiers,[2] two sharpshooters (revolver) and three others who had failed to qualify as marksmen. These preliminary experiments encouraged us to carry out more extensive trials with large numbers of men and improved apparatus. With the coöperation of the office of the Surgeon General, we were able to test seventy-three men of known marksmanship at Camp Meade. In calculating the coefficient of correlation between rifle shooting ability and steadiness we used the rank difference method. This gave us a positive correlation of 0.61. Since this correlation is well within the significant and practically useful region, it may prove to be of value in selecting *a,* men physiologically unfitted to become rifle shots; and *b,* men who show an unusual degree of motor control and are therefore preferred material for intensive range training. The advantages of improving motor control and therefore shooting ability by practice with the steadiness apparatus rather than with firearms will be discussed further on.

Apparatus. Our preliminary experiments were carried out according to Whipple's directions (pp. 156–157), but we soon found several serious practical objections to this method. The Whipple apparatus consists of a brass plate set at an angle of 45° and pierced with two series of holes whose diameters, in sixty-fourths of an inch, are 32, 20, appears when the needle is first inserted that is almost immediately checked by the subject's own control. The time for each trial was 20 seconds. We found no difficulty in giving the test at the rate of twenty men per hour.

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[1] Whipple, G. M., 1914, Manual of mental and physical tests, Baltimore, Warwick & York, Inc.

[2] We are indebted to Col. O. G. Brown for placing these men at our disposal.

Tests made with two groups of students during the past 2 years had shown that hole number 13 is a satisfactory starting point. In the majority of cases each man was therefore tested in holes 13, 12, 11 and 10. When the subject made a total of less than 50 contacts in these four holes he was also tested in number 9. Preliminary experience had shown that when the total number of contacts for any series of holes is greater than about 50 it is generally useless to make observations in the next smaller hole, since the contacts then tend to become so frequent that an accurate count is impossible. We therefore adopted this principle with all subjects. In six cases the subject could not get beyond hole number 11; and the most unsteady man, who was likewise the poorest marksman, made 88 contacts in hole number 13. (See table 3.) It is of interest that this individual was the only subject of more than one hundred tested who made more than 50 contacts in hole 13. This fact seems completely to justify our selection of hole 13 as a practical starting point for the test.

An analysis of rifle shooting. Before discussing our method of scoring the steadiness records and their correlation with marksmanship, a brief analysis of the physiological and psychological aspects of rifle shooting seems in order. Successful rifle shooting requires: *a*, vision sufficiently good to see the sights and target clearly and without blurring; *b*, a high degree of motor control (eye and hand coördination) to enable the marksman to hold the target, the front and the rear sights in alignment; *c*, a slow, steady pressure, not a jerk, on the trigger, the so-called "trigger-squeeze," applied at the moment of sight and target alignment; and *d*, a complete indifference to the sound of the shot, that is, an absence of so-called "gun-shyness" or "flinching." Such technical points as sight elevation and judgment in the matter of cross wind currents need not concern us in this discussion.

Of these four factors the first, vision, can in most cases be corrected with proper glasses. The trigger-squeeze is a matter of practice and presents no insurmountable physiological or psychological obstacle. A "gun-shy" individual, whether man or dog, is generally considered hopeless, since the response is ordinarily quite beyond voluntary control. Probably, in the case of man, a study and exposure of the origin of the gun-shyness would remove the difficulty far more rapidly than attempting to accustom the individual to the sound of gunfire. As a practical problem, however, gun-shy individuals are relatively rare. In our study we found a single man who made an excellent steadiness record but failed to qualify as a marksman. (See number 66 in table 3, column 1. This individual has the largest D^2 value also.) We suspect this man of being gun-shy but we have no positive evidence for our suspicion.

There remains the factor of motor control. Although an individual with an oscillating gun barrel is at an obvious disadvantage on the rifle range, neither the degree of involuntary tremors nor the control of such tremors has ever received systematic study in the case of marksmen. In our opinion, involuntary movements constitute an important and easily measured barrier to successful marksmanship. It must remain for future investigations to show whether training of involuntary movements by the use of a steadiness apparatus will improve rifle shooting. The practical advantages of preparing men to become marksmen by laboratory training rather than by range practice are too obvious to require further comment.

The rating of marksmanship. Rifle shots in the Infantry of the United States Army are divided into six groups, as shown in the following table.

TABLE 1

GRADE	RIFLE FIRING POINTS	NUMBER OF MEN TESTED
1. Expert rifleman	253	3
2. Sharpshooter	238	17
3. Marksman	202	14
4. First-class man	177	12
5. Second-class man	152	10
6. Unqualified	Less than 152	17

Although all men in the first five grades must attain a high degree of accuracy in estimating distances, the main criterion for ranking rifle shots is actual range score. In our table 3 we have therefore ranked our seventy-three subjects on the basis of their best range scores, made under uniform and carefully controlled conditions. The exact details of distances, number of shots fired, method of scoring targets, etc., are irrelevant to our discussion but may be found in the Small Arms Firing Manual for 1913, issued by the War Department.

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Method of scoring the steadiness test. In our laboratory experience with student subjects, we found that the steadiest individuals could hold the stylus for 20 seconds in holes 13, 12 and 11 without a single contact. As an empirical basis for comparison, we therefore counted all contacts in hole number 10 as single units, but penalized contacts in 11, 12 and 13 by counting single contacts as 5, 10 and 20 units respectively. The test was so difficult in hole number 9 that in this case two contacts were counted as one unit. The total number of units was then added and divided by the number of holes tested (3, 4 or 5) which gave a steadiness score for a comparative rating. The exact method of scoring with three records taken from our observations is shown in table 2.

TABLE 2

Method of scoring the steadiness records

HOLE NOS.	CONTACTS	UNITS	SCORE
Subject 1			
13	0	× 20.0 =	0
12	0	× 10.0 =	0
11	2	× 5.0 =	10
10	3	× 1.0 =	3
9	14	× 0.5 =	7
Total	19		$\frac{20}{5}=4$
Subject 19			
13	2	× 20.0 =	40
12	3	× 10.0 =	30
11	10	× 5.0 =	50
10	22	× 1.0 =	22
9	38	× 0.5 =	19
Total	75		$\frac{161}{5}=32.2$
Subject 47			
13	4	× 20.0 =	80
12	11	× 10.0 =	110
11	19	× 5.0 =	95
10	32	× 1.0 =	32
9			
Total	66		$\frac{317}{4}=79.2$

TABLE 3

Correlation of marksmanship and steadiness by rank differences

MARKSMANSHIP		STEADINESS		D^2	MARKSMANSHIP		STEADINESS		D^2
Rank	Score	Rank	Score		Rank	Score	Rank	Score	
1.0	266	1	4.0	0.00	38.0	196	34	55.5	16.00
2.0	264	16	27.9	196.00	39.0	193	59	140.7	400.00
3.0	253	25	39.2	484.00	40.0	190	10.5	16.0	870.25
4.0	248	14	25.5	100.00	41.0	189	43	66.5	4.00
5.0	247	5	13.6	0.00	42.0	188	58	135.2	256.00
6.0	244	38	60.5	1024.00	43.0	186	50	88.2	49.00
7.0	242	37	58.6	900.00	44.0	182	66	195.0	484.00
8.0	241	8	15.1	0.00	45.0	179	30	44.5	225.00
9.0	240	60	143.5	2601.00	46.0	178	4	13.1	1764.00
11.0	239	19	32.2	64.00	47.0	176	56	118.2	81.00
11.0	239	12	17.3	1.00	48.0	173	54	108.0	36.00
11.0	239	36	58.4	625.00	49.0	171	31	49.2	324.00
16.5	238	6	14.4	110.25	50.0	169	63	164.0	169.00
16.5	238	21	34.2	20.25	51.0	168	42	66.2	81.00
16.5	238	13	18.3	12.25	52.0	163	62	153.2	100.00
16.5	238	39	61.7	506.25	53.0	158	67	240.0	196.00
16.5	238	15	27.5	2.25	54.0	156	46	76.5	64.00
16.5	238	22	36.5	30.25	55.0	154	35	57.5	400.00
16.5	238	41	65.2	600.25	56.0	153	55	111.5	1.00
16.5	238	2	7.9	210.25	57.0	145	68	270.2	121.00
21.0	230	49	83.2	784.00	58.0	140	51	94.7	49.00
22.0	229	29	44.4	49.00	59.0	137	64	164.5	25.00
23.5	225	32	50.2	72.25	60.0	135	40	64.8	400.00
23.5	225	28	41.5	20.25	61.0	133	17	30.5	1936.00
25.0	218	26	40.2	1.00	62.0	124	71	335.0	121.00
26.0	215	24	39.0	4.00	63.0	122	53	107.7	100.00
27.5	213	23	38.0	20.25	64.0	114	70	316.6	36.00
27.5	213	44	71.7	272.25	65.0	112	33	51.5	1024.00
29.5	209	9	15.6	420.25	66.0	100	10.5	16.0	3080.25
29.5	209	48	79.5	342.25	67.0	90	72	580.0	25.00
31.0	205	27	40.5	16.00	68.0	76	47	79.2	441.00
32.5	204	18	31.6	210.25	69.0	75	69	295.0	0.00
32.5	204	7	14.6	650.25	70.0	67	57	125.5	169.00
34.0	203	52	100.7	324.00	71.0	61	65	186.0	36.00
35.0	199	45	72.7	100.00	72.0	44	61	149.5	121.00
36.5	197	3	11.9	1122.25	73.0	26	73	1280.0	0.00
36.5	197	20	33.2	272.25					

$\Sigma D^2 = 25372.00$

For 73 cases $\rho = +0.61$

The coefficient of correlation between rifle shooting and steadiness. We were interested in determining the degree to which great steadiness and expert shooting ability tend to occur in the same individual. We therefore calculated our coefficient of correlation between rifle shooting and steadiness by the rank difference method.[6]

The formula:

$$\rho = 1 - \frac{6 \Sigma D^2}{n (n^2 - 1)}$$

gives us a value for $\rho = +0.61$. In addition to each man's rank, we have given the actual rifle and steadiness scores in our table 3.

Where several men had the same score as the three men who made 239 on the rifle range (number 11, table 3) they were all given the same rank (11) and in this instance rank numbers 10 and 12 were not assigned. This is the customary statistical procedure. Although the high positive value of the coefficient of correlation is well within the significant range, it must not be imagined that a single expert rifle shot will inevitably make a high steadiness score. Predictive tests are useful as short cuts in grouping large numbers of individuals. They are incomparably more efficient, provided the tests themselves have been properly tested, than the methods of trial and error and learning by experience.

CONCLUSIONS

From the foregoing experiments it is clear that steadiness is definitely associated ($\rho = +0.61$) with marksmanship in the case of men who have had range experience. The practical value of a test of this sort lies in its use as a predictive means of selecting probable expert rifle material and eliminating men who are physiologically unfitted to qualify as marksmen. Since the steadiness test requires only about 3 minutes per man, it would be far more efficient as regards time, cost of munitions and the nerves of men and officers to select material for elimination or range development by this test rather than by the slow and costly method of giving every man a try-out with the rifle. In our experiments we have, however, only tested the test on men of known ability. It remains for future work to show how extensively this test may be used with raw recruits. Range practice may increase a man's steadiness and, conversely, steadiness practice may improve a man's range score. Further experiments alone will tell.

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[6] The tables published by the Scott Co., Philadelphia, in the Journal of Applied Psychology, iv, 115, greatly facilitate the computation of the coefficient of correlation by the rank difference method.

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A METHOD FOR DETERMINING THE FINER DUST PARTICLES IN AIR *

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IT is well known that a suspension may be removed from air by bringing the air contact with water. The more intimate nd prolonged the contact, the more effi-ient will be the removal. One of the implest means of effecting such an inti-ate mixture, for purposes of dust anal-sis, is to introduce a small volume of air to a syringe containing some water. The yringe may then be shaken vigorously and very thorough mixture of air and water ccomplished. If a drop of the water be xamined with the microscope by ordinary lumination, very few particles, if any, will e seen; by oblique illumination, however, e minute matter scintillates in the field nd may readily be counted. This con-itutes, in brief, the principles of the ethod presented in this paper, a method at is concerned chiefly with the minute articles.

In E. V. Hill's method (1) the air is drawn through e narrow nozzle of a capsule attached to a metal ringe; whereupon some of the particles impinge pon a small glass slip covered with a thin layer of a ansparent adhesive mixture. A variable percentage particles is actually caught, owing partly to the ct that the particles differ in inertia. The greater e inertia, the greater the likelihood of their being irected against the adhesive surface. Because of is property of inertia, the rate with which the iston of the syringe is withdrawn will influence the sult. The count is made with ordinary illumina-on.

Palmer's method (2) consists in the filtration of r by means of the water-spray; but here again the moval is far from complete. Katz, Longfellow, and ieldner (3) find that the Palmer apparatus retains out 45 per cent. by weight of air-floated silica and per cent. of tobacco smoke as measured by the yndall effect. The air passing at the rate of 4 cubic feet per minute is in contact with the water but a very short time.

Aitken devised a method for the estimation of atmospheric dust (4) based on the principle that the dust particles serve as nuclei for the condensation of water-vapor. In this case all particles, even the minutest or those of an ionic nature, are included in the precipitation and share in the count. The watery envelope makes any determination of size difficult. The coalescence and evaporation of droplets may easily give rise to erroneous counts. It is a method requiring extraordinary care and skill.

Bill's electric precipitation method (5) is in process of development. The removal of dust particles appeared to be greater than in Palmer's method but in its present form it is unsuitable for field work. In this method, as in Aitken's, all particles of whatever size tend to undergo precipitation. The determination is gravimetric.

PROCEDURE

A Luer syringe graduated to 100 c. c. but having a capacity of about 160 c. c. is thoroughly cleaned with soap and water and alcohol to remove all grease. It is then rinsed with freshly distilled water and filled with distilled water free from air bubbles. After forcing out all the water, 20 c. c. of distilled water are taken into the syringe from an Erlenmeyer flask provided with a cotton-wool filter (Figure 1). The syringe is now ready for the air sample. The piston is withdrawn sufficiently to admit approximately 100 c. c. The exact volume may be read from the graduation. While placing the finger tightly against the nozzle, a piece of rubber membrane intervening, the syringe is shaken vigorously for one minute with an up and down motion. With the syringe in a vertical position the water is brought up to the very tip of the nozzle and then withdrawn slightly to

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admit a small bubble of air. This manipulation is intended to catch the particles adherent in the narrow portion of the nozzle and bring them into suspension.

The next step consists in allowing a drop to flow from the syringe into the chamber of a Levy blood counter. This is best done with the metal connecting piece on the nozzle. The particles are counted by oblique illumination. The results reported in this paper were obtained with the No. 3 objective of a Leitz microscope with a circular piece of blue glass below the Abbe condenser. A 75-watt Mazda daylight lamp was placed in front of the mirror, a jar of water intervening to absorb the heat rays. Both lamp and jar were covered with an asbestos hood.

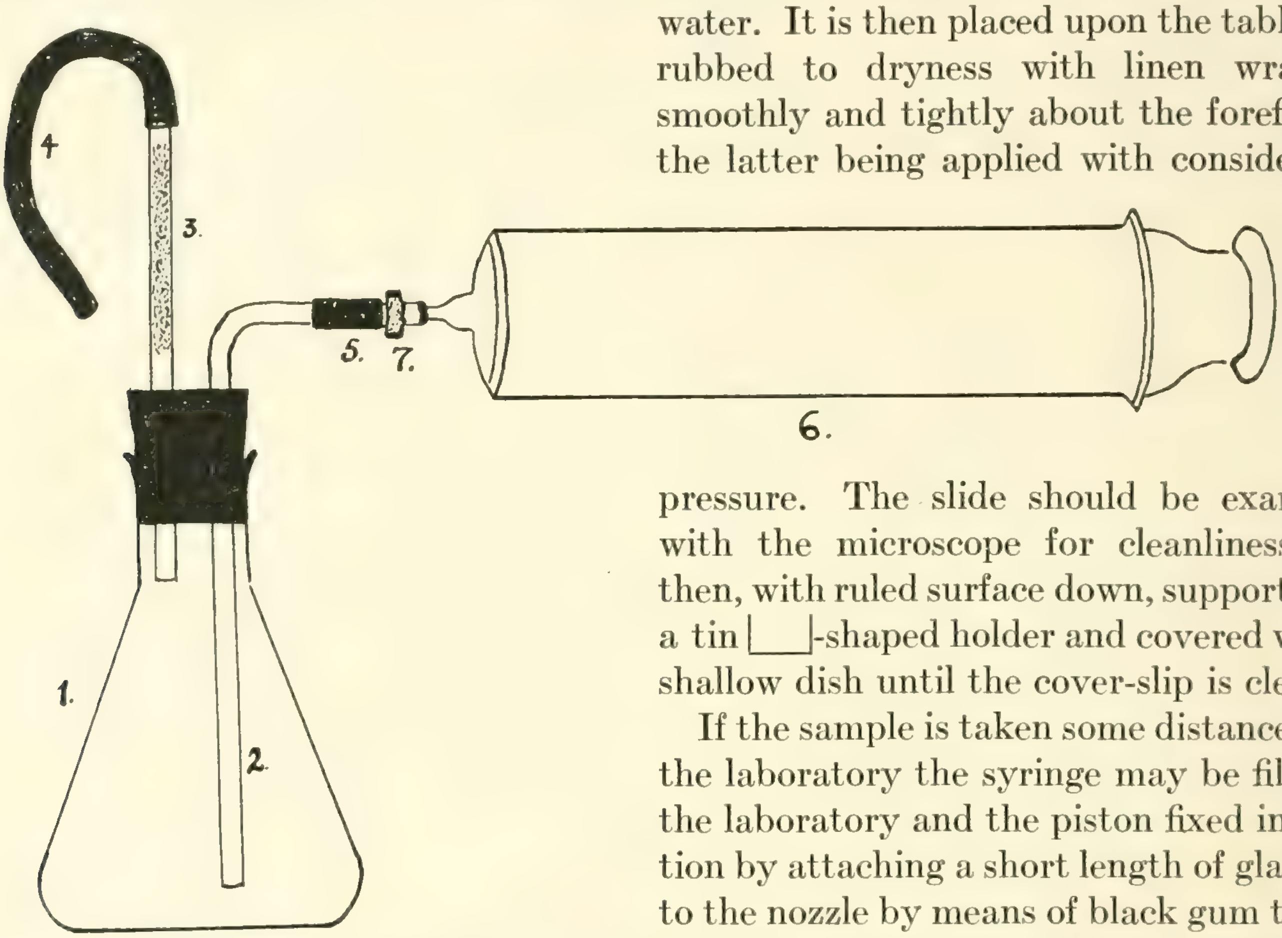

Fig. 1. — 1. Erlenmeyer flask. 2. Glass tube. 3. Glass tube containing cotton-wool. 4. Rubber tubing. 5. Pure gum connecting piece. 6. Luer syringe. 7. Metallic connecting piece.

It will require patient practice in the beginning to free the ruled surface and cover of the Levy chamber of dust particles. A surface sufficiently clean for ordinary illumination will not do at all for oblique illumination. It is well to have on hand several handkerchiefs of pure linen that have been washed several times. Lens paper is useless. The ruled surface is thoroughly rubbed with the thumb, under a tap of warm water, and finally rinsed for a moment in hot water. It is then placed upon the table and rubbed to dryness with linen wrapped smoothly and tightly about the forefinger, the latter being applied with considerable pressure. The slide should be examined with the microscope for cleanliness and then, with ruled surface down, supported on a tin ⊔-shaped holder and covered with a shallow dish until the cover-slip is cleaned.

If the sample is taken some distance from the laboratory the syringe may be filled in the laboratory and the piston fixed in position by attaching a short length of glass rod to the nozzle by means of black gum tubing free from powder. After the sample has been taken, it remains in the syringe with the piston fixed. This permits another thorough mixture on reaching the laboratory. The entire number of particles in two fields, each a square millimeter, are counted. The average of these two counts is used in the calculation. Those fields are selected which are divided into sixteen smaller areas, each a sixteenth of a square millimeter. The particles must be allowed to settle before the count is made.

A Modification of the Foregoing Procedure

A few determinations have been made with a modification of the above method, somewhat more complicated, but based on exactly the same principle. In this paper the steps will be merely indicated.

A test tube of 150 c. c. capacity, provided with a glass stopper perforated by two glass tubes, one of which extends to the bottom, is filled with distilled water. A filter (Figure 2) is connected with the short tube. The syringe being attached to the long tube, the contents of the test tube are emptied to the 30 c. c. mark. The test tube now contains filtered air and 30 c. c. of distilled water. An air sample may be taken by removing the filter and connecting the short tube with the syringe. After vigorously shaking the test tube, the syringe is again connected with the long tube for the purpose of bringing into suspension all particles adherent in the long tube. This is done by moving the piston back and forth a short distance. Finally, a portion of the water is allowed to remain in the syringe so that a drop may be transferred to the counting chamber.

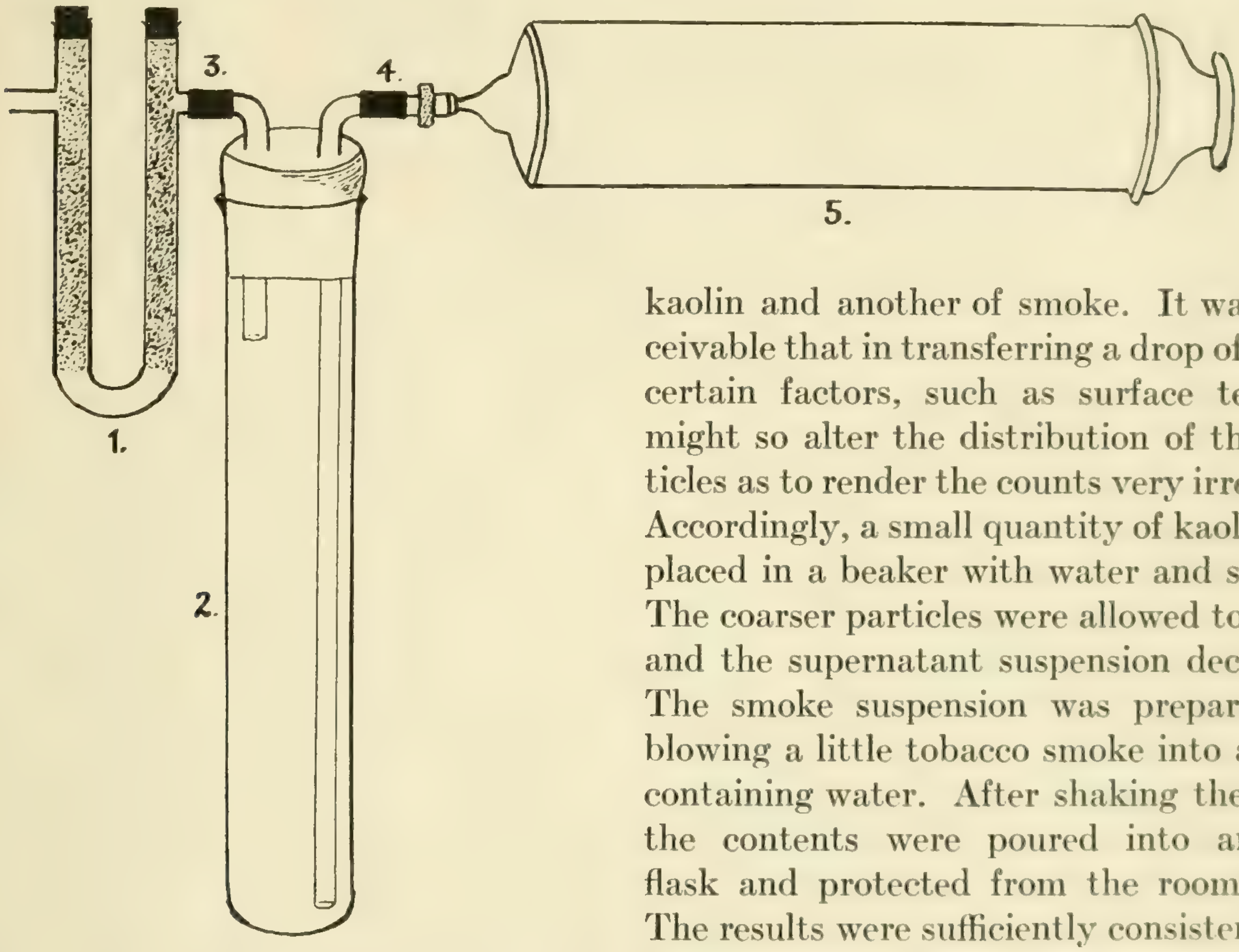

Fig. 2. — 1. Filter containing cotton-wool. 2. Test tube provided with glass stopper perforated by two glass tubes. 3 and 4. Pure gum connecting pieces. 5. Luer syringe.

Results

Before proceeding with the actual determination of particles present in air, it appeared desirable to test the consistency of the method with a prepared suspension of kaolin and another of smoke. It was conceivable that in transferring a drop of water certain factors, such as surface tension, might so alter the distribution of the particles as to render the counts very irregular. Accordingly, a small quantity of kaolin was placed in a beaker with water and stirred. The coarser particles were allowed to settle and the supernatant suspension decanted. The smoke suspension was prepared by blowing a little tobacco smoke into a flask containing water. After shaking the flask, the contents were poured into another flask and protected from the room dust. The results were sufficiently consistent and clearly indicated that no factors operate to introduce serious irregularity in this part of the procedure.

Counts made with distilled water alone — not prepared with unusual care — showed an average number of particles per square millimeter of 12 ± 2. This figure is used as a correction in calculating the number of particles per unit volume of air. The distilled water is not entirely responsible for this count; some particles unavoidably remain on the ruled surface during the cleaning process.

The results of the determinations of the dust content of outdoor air and laboratory air appear in Table 1. The outdoor samples were obtained in a street comparatively free of traffic; those of indoor air were taken in one of the rooms of the laboratory

TABLE 1.—DUST PARTICLES IN THE AIR

Date	Source of Sample	Number of Sample	Size of Sample (c.c.)	Time	Count per Sq. Mm.		Difference	Average Count per Sq. Mm.	Particles per c.c. of Air
					1st Field	2d Field			
October 30......	outdoors	1	103	10:30	34	28	6	31	36,800
		2	202	11:10	40	50	10	45	32,600
November 1.......	"	1	202	1:30	31	29	2	30	17,800
		2	306	2:05	38	50	12	44	20,900
		3	405	3:50	53	60	7	57	22,200
November 3......	"	1	205	9:45	55	54	1	55	41,900
		2	207	11:00	52	51	1	52	38,600
November 4......	"	1	102	10:30	33	38	5	36	47,000
		2	202	11:40	59	53	6	56	43,500
		3	205	4:40	50	53	3	52	39,000
November 5......	"	1	205	1:42	43	53	10	48	35,000
		2	302	2:50	58	56	2	57	29,500
November 8......	"	1	306	10:30	64	68	4	66	35,200
		2	203	11:50	43	44	1	44	31,500
		3	101	1:25	27	30	3	29	33,600
		4	205	2:40	39	51	12	45	32,100
		5	100	3:50	31	31	0	31	38,000
		6	102	4:30	25	32	7	29	33,300
January 5......	"	1	102	9:35	47	63	19	57	88,200
		2	202	9:42	92	103	11	98	85,100
		3	207	9:47	96	101	5	98	82,600
October 28......	laboratory	1	105	10:45	41	39	2	40	53,300
		2	102	11:30	38	35	3	37	49,000
		3	102	1:00	34	36	2	35	45,000
November 9......	"	1	204	10:30	67	70	3	69	55,800
		2	202	11:09	73	67	6	70	57,400
		3	202	1:15	68	62	6	65	52,400
		4	202	2:20	60	60	0	60	47,500
		5	204	3:00	71	53	18	62	50,000
		6	202	3:50	64	65	1	65	52,400
November 10......	"	1	205	10:05	53	43	10	48	35,100
		2	206	1:00	56	52	4	54	40,700
December 28......	"	1	206	1:00	62	70	8	66	52,400
		2	206	2:20	61	51	10	56	42,700
		3	205	3:35	67	54	13	61	47,800
December 31......	"	1	102	10:45	37	49	12	43	60,700
		2	102	11:15	45	31	14	38	50,900
January 4......	closed cabinet	1	102	1:10	31	34	3	33	41,100
		2	101	2:05	33	35	2	34	43,500

in which there was generally but one occupant. If the dust content of the air were absolutely constant, our method would not permit us to expect a better agreement than the figures in the table actually show. With one or two exceptions, the average deviation of the number of dust particles per cubic centimeter of air is not more than ±4,000 for 100 c.c. samples, nor more than ±2,000 for 200 c. c. samples. The derivation of these figures will readily be understood upon recalling that the average count for distilled water is 12 ±2. Owing to the small number of samples in most of the series, there is no significance in the fact that in one or two instances the above limits are slightly exceeded.

The results in the table, therefore, mean that the amount of dust present in the air on any single day was, in general, practically constant under the conditions that prevailed when the samples were taken. When one bears in mind the errors incident to enumeration and the possibility of certain influences disturbing the dust content of the air, the agreement is remarkable. There is a variation from day to day and the figures for indoor air are on the whole higher than those for outdoor air, although they are not strictly comparable, having been obtained on different days. On the morning of January 5, the atmosphere was unusually smoky and the counts are correspondingly high. The samples of air from a closed cabinet free from disturbing influences are practically identical in their dust content.

DISCUSSION

Before the method was actually tested, it seemed doubtful whether one could hope for consistent results, partly because of the possibility of the inclusion of particles between the barrel and piston of the syringe and partly because of the exposure of the piston to the dust particles of the air. But experience showed that it makes no difference whether samples of 100, 200, or 300 c. c. are taken; in other words, whether the piston is withdrawn once, twice, or three times. These factors, then, if they operate, do not endanger the usefulness of the method. In the modified form of the method any error from this source would disappear.

One minute's shaking is sufficient to bring the particles into aqueous suspension. A more prolonged shaking does not increase the count. This is exactly what one would anticipate from the work of Katz, Longfellow, and Fieldner (3) who find that Palmer's apparatus in which the air is in contact with the water for a very brief interval retains 13 per cent. of smoke.

The same objection applies to this method as applies to all methods in which water is used as a medium. Water-soluble particles will not be included in the count. In many cases, however, particles lost in solution may be determined by chemical methods. Bill (5) points out that the particulate matter in the Palmer suspensions shows a tendency to form small masses or clumps. The minute particles in my own suspensions have not shown any agglomeration. Counts made of suspensions that were allowed to stand for nearly two hours were practically as high as those made in the beginning.

In routine work, such as the examination of air in factories, where several samples taken at short intervals are desired, the contents of the syringe may be emptied into small 25 c.c. Erlenmeyer flasks and covered with rubber membrane secured by an elastic band. In the modified form of the method, a series of test tubes each provided with a glass stopper perforated by two glass tubes (Figure 2) may be employed.

CONCLUSION

1. A method has been presented in the foregoing pages, based on the enumeration, by oblique illumination, of minute particles in a sample of air brought into aqueous suspension by shaking the sample with water.

2. Success in the use of the method demands strict attention to cleanliness.

3. The method requires but a very small sample of air and yields consistent results.

4. The method ought to be especially suitable for the determination of smoke particles and should also prove useful in determining the efficiency of air-filtering devices in ventilation systems.

BIBLIOGRAPHY

1. Hill, E. V.: Quantitative Determination of Air Dust. Heating and Ventilating Magazine, 1917, **14**, 23.
2. Palmer, G. T.: A New Sampling Apparatus for the Determination of Aërial Dust. Am. Jour. Pub. Health, 1916, **6**, 54.
3. Katz, S. H., Longfellow, E. S., and Fieldner, A. C.: Efficiency of the Palmer Apparatus for Determining Dust in Air. Jour. Indust. Hyg., 1920–1921, **2**, 167.
4. Aitken, J.: On the Number of Dust Particles in the Atmosphere. Trans. Roy. Soc. Edin., 1889, **35**, 1.
5. Bill, J. P.: The Electrostatic Method of Dust Collection as Applied to the Sanitary Analysis of Air. Jour. Indust. Hyg., 1919–1920, **1**, 323.

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